

Figure 1

# TRAIL induced apoptosis in Jurkat Cells

12 HOURS

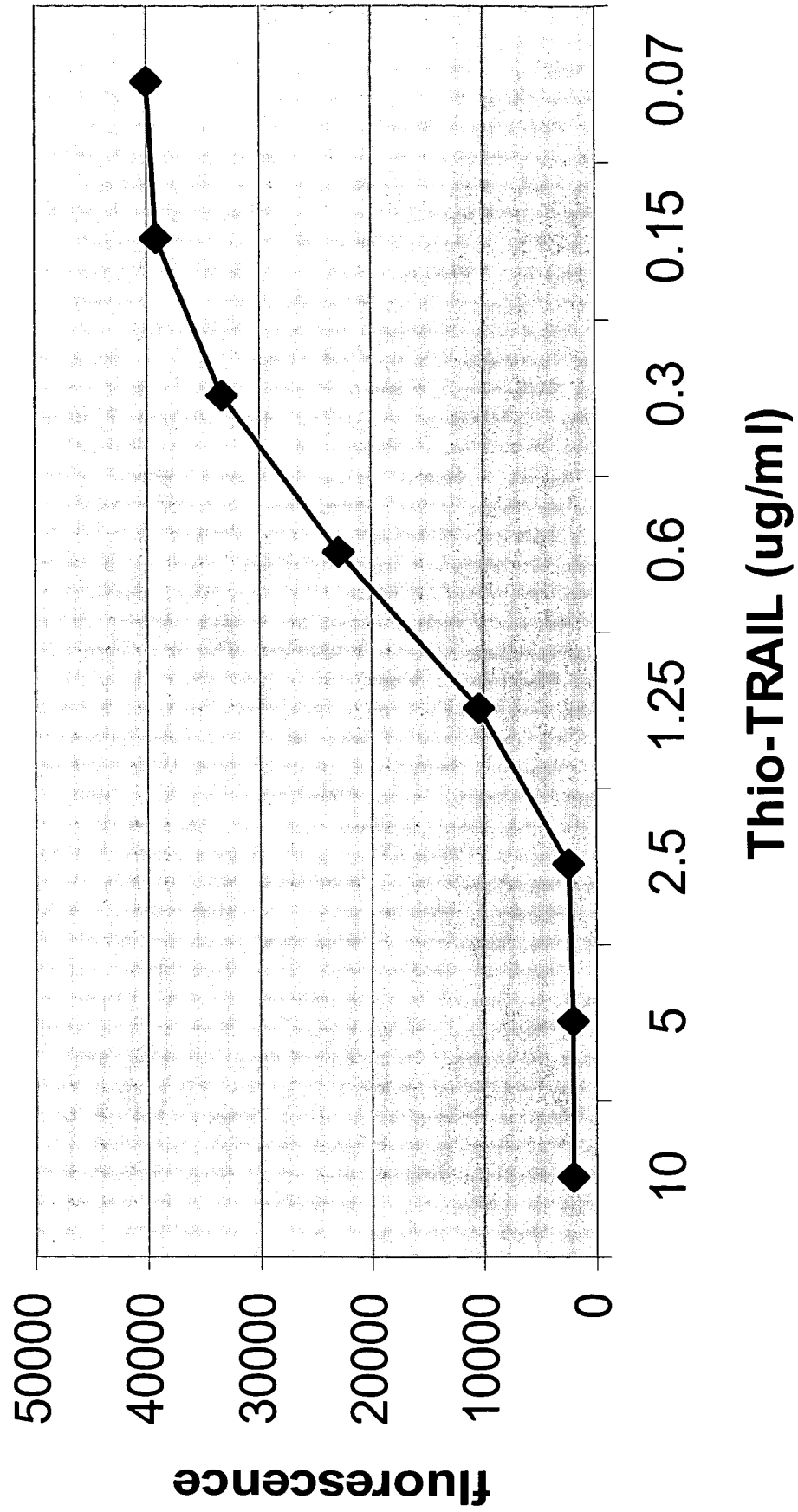
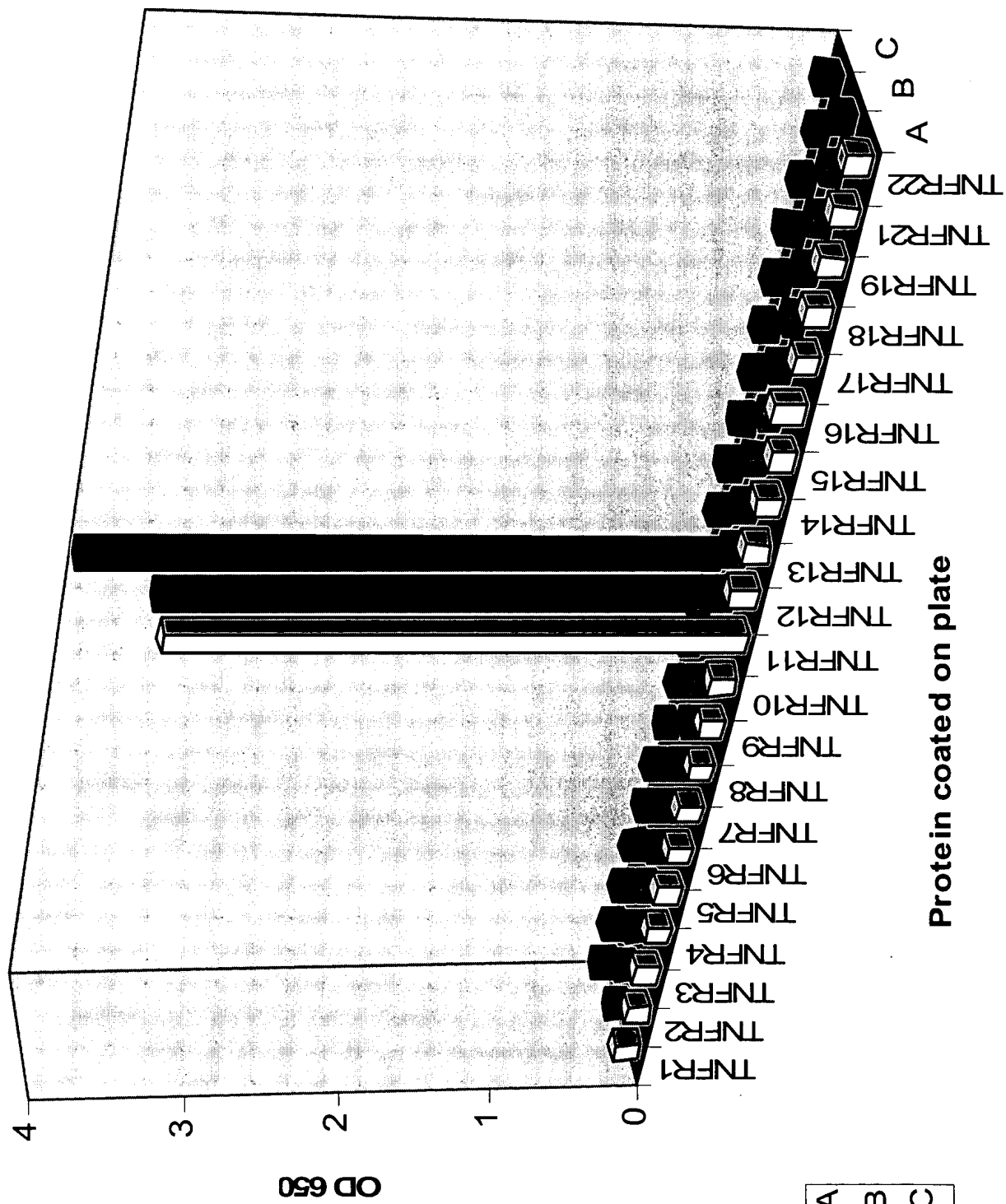


Figure 2

# Specificity of DR5 Functional Antibodies



Effect of 3 Different DR5 Antibody Agonists On Jurkat Cells

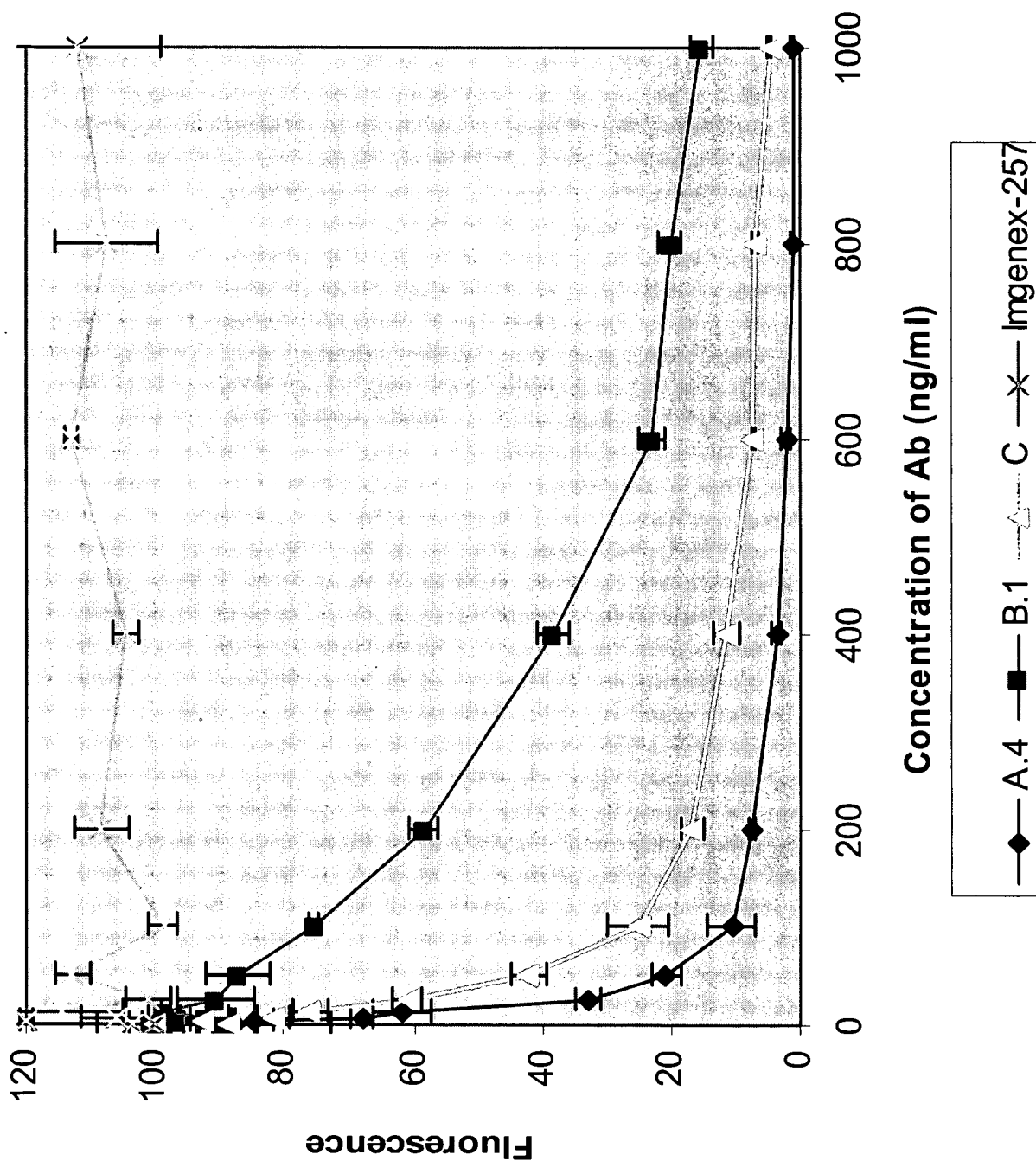
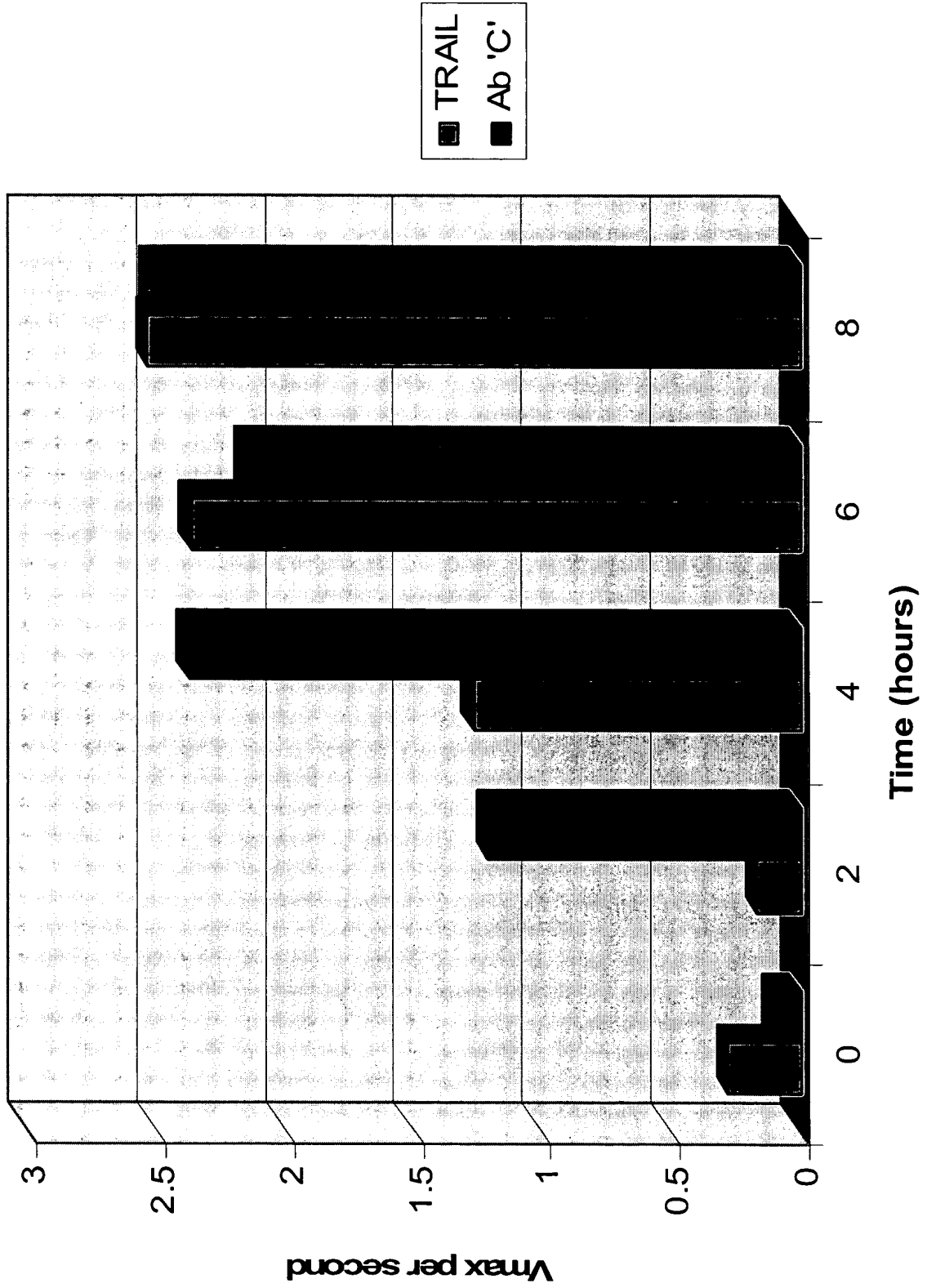


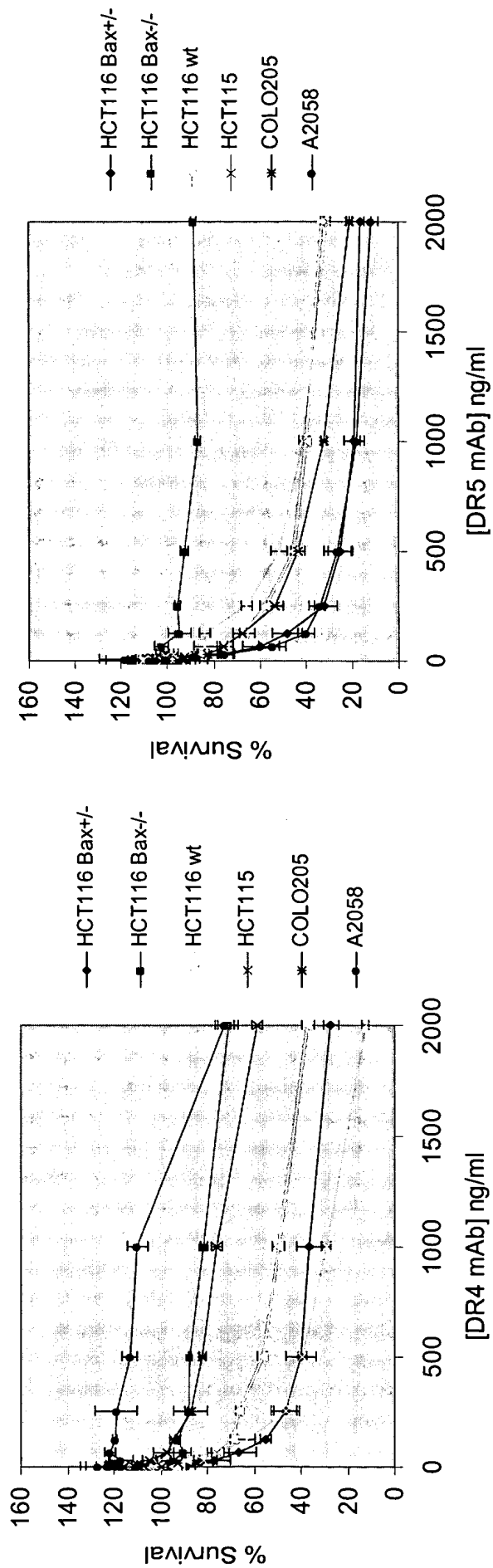
Figure 4

Caspase 3 activation in treated Jurkat cells

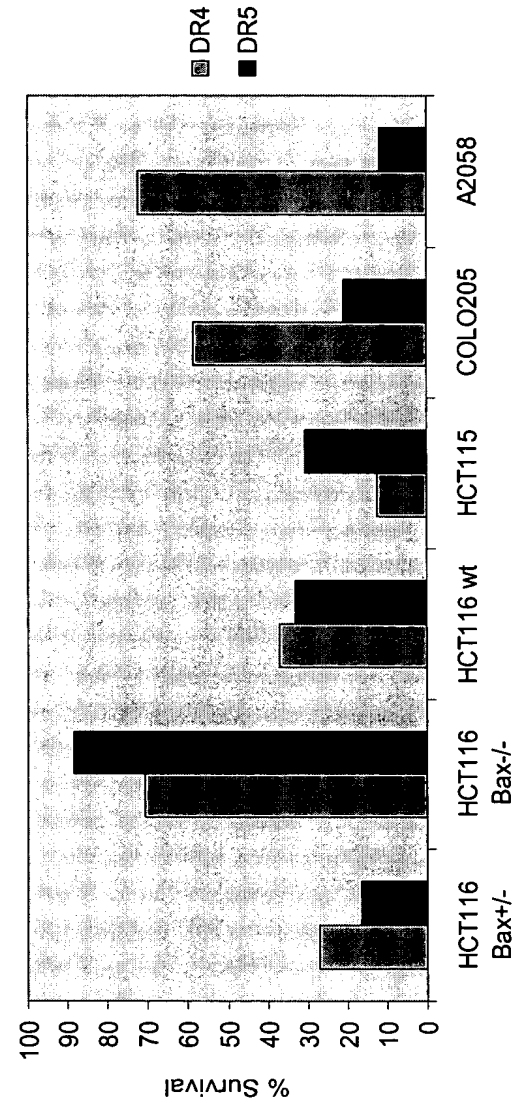


# Figure 5

Effect of DR4 / DR5 Functional Antibodies on Colon and Melanoma Cancer Cell Lines



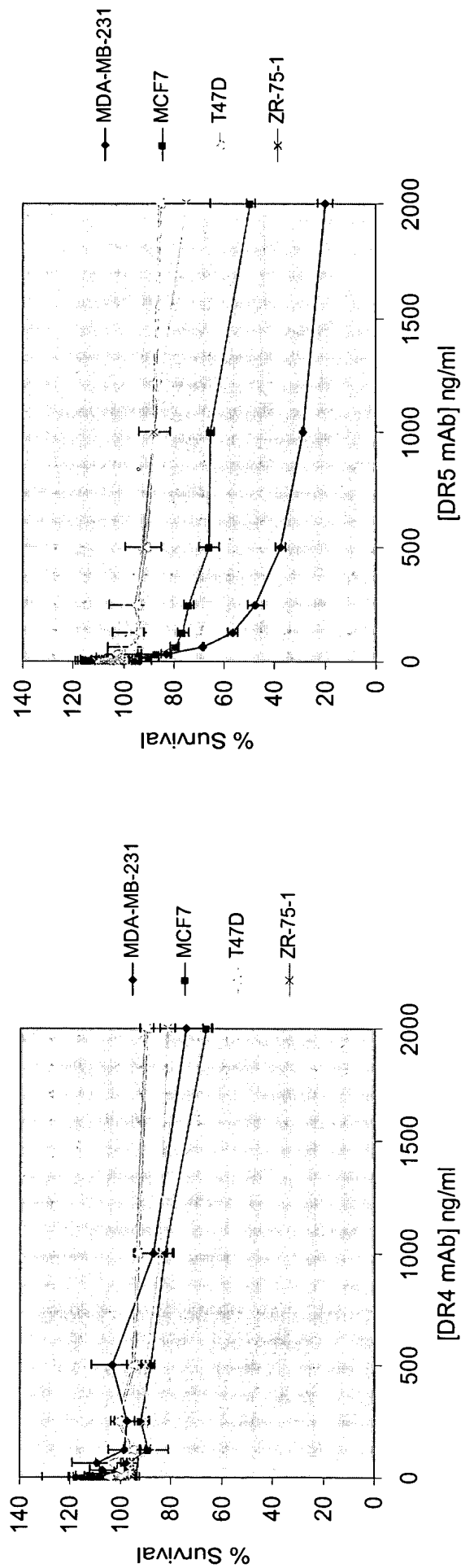
Effect of 2ug/ml Antibody on Cell lines



BEST AVAILABLE COPY

# Figure 6

Effect of DR4 / DR5 Functional Antibodies on Breast Cancer Cell Lines



Effect of 2ug/ml Antibody on various cell lines

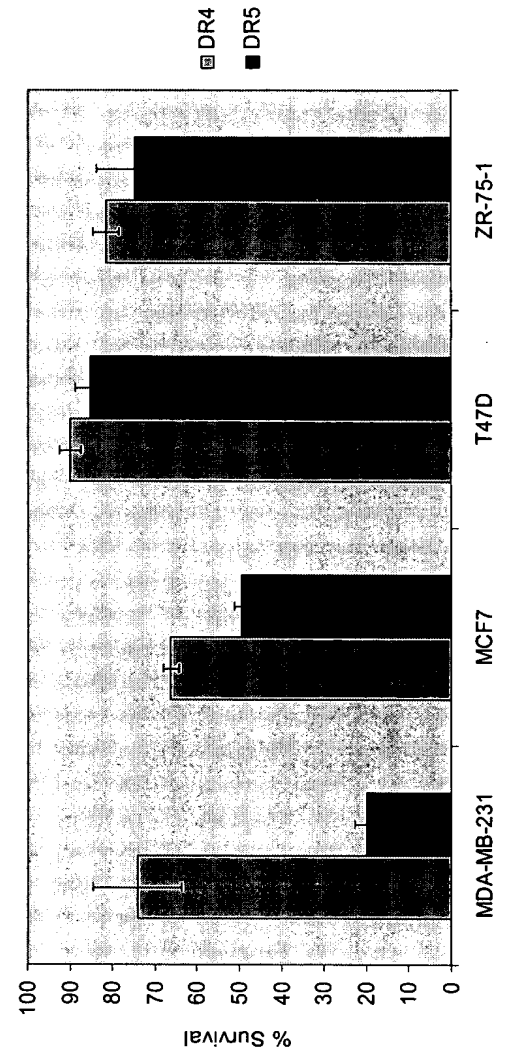


Figure 7

# **Dose Response To DR5 Antibody Agonist** **Normal vs Tumor Cells**

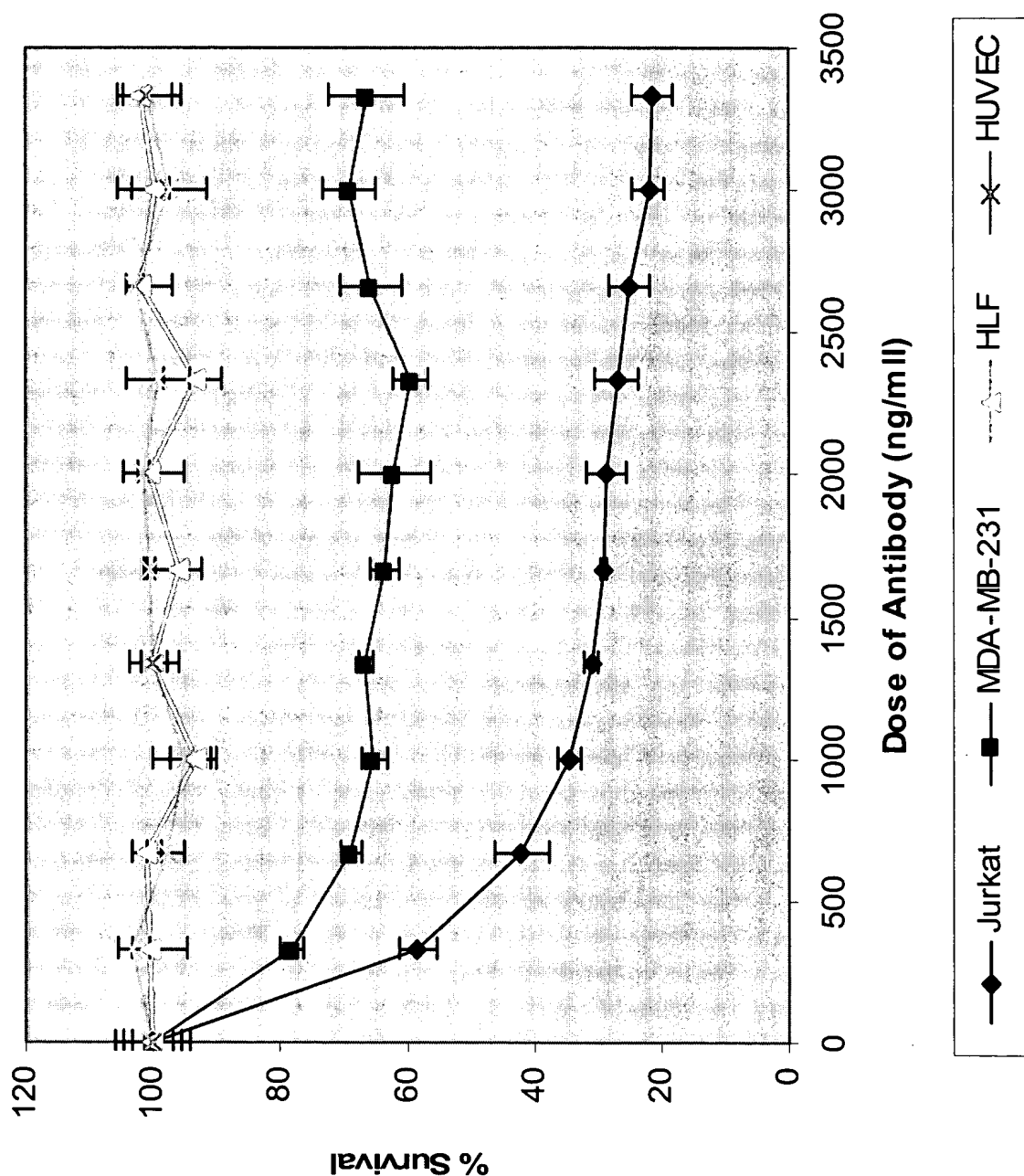


Figure 8

**DR5 Antibody Agonist "A" - Caspase 3 Activation**

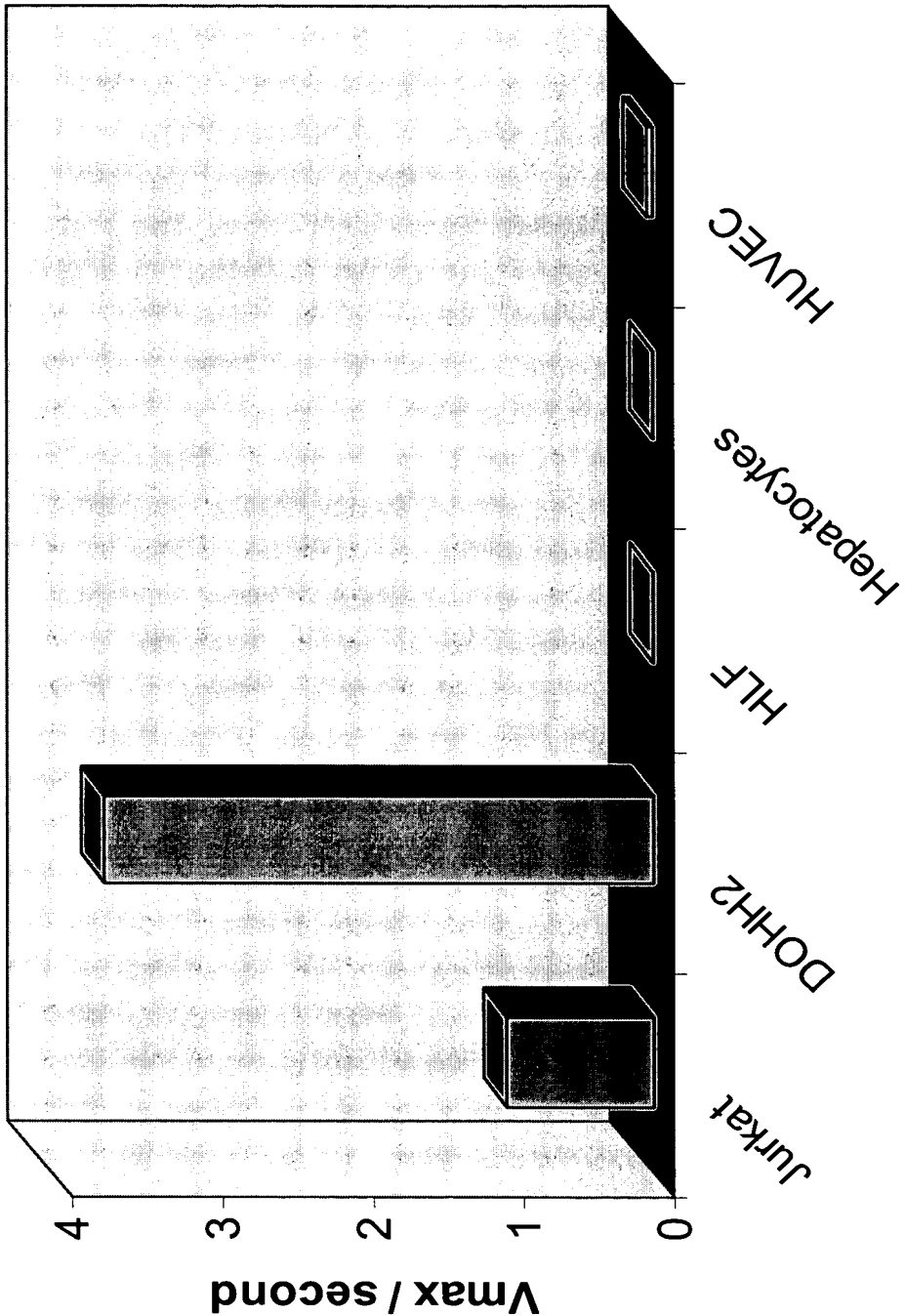




Figure 9

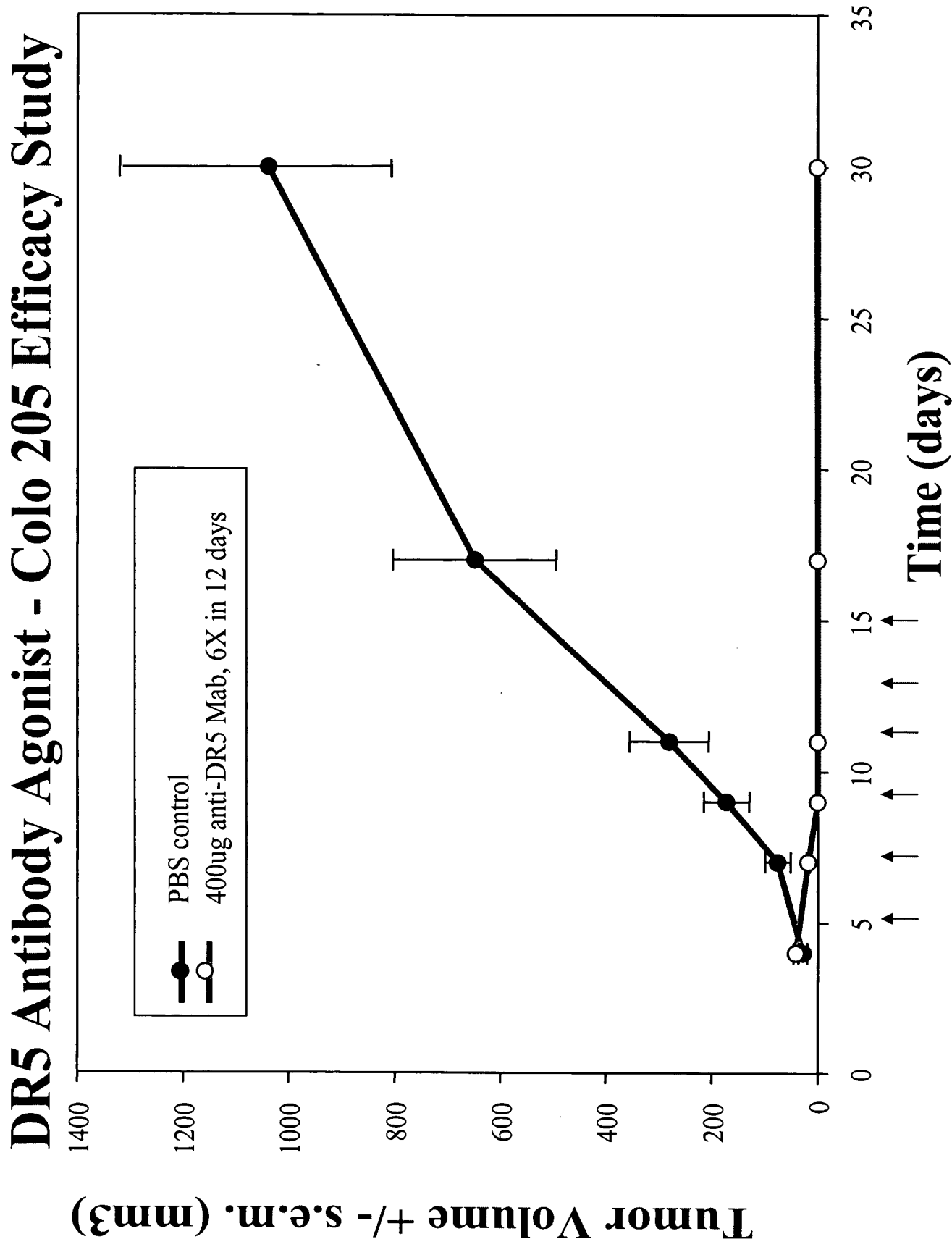


Figure 10

# Anti-DR5 Dose Response, COLO205 Subcutaneous Model

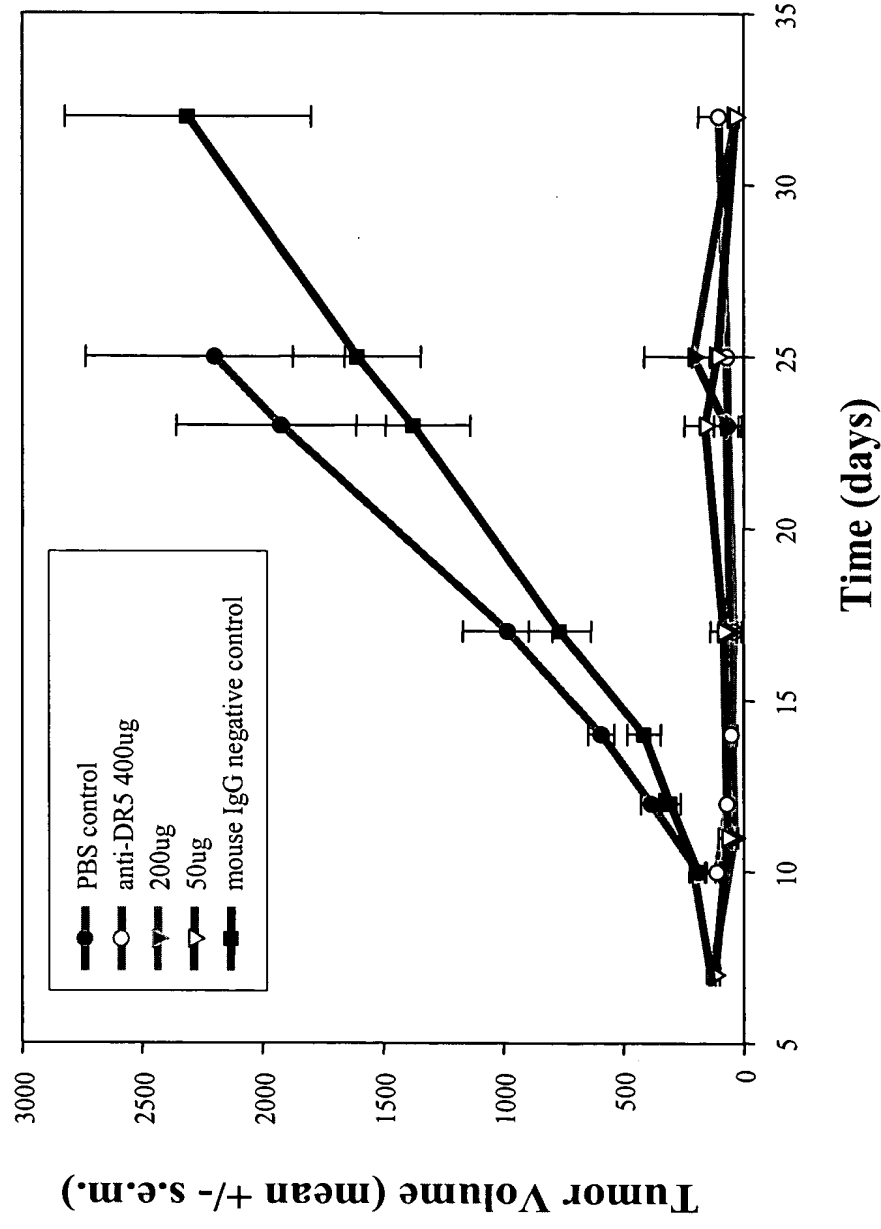
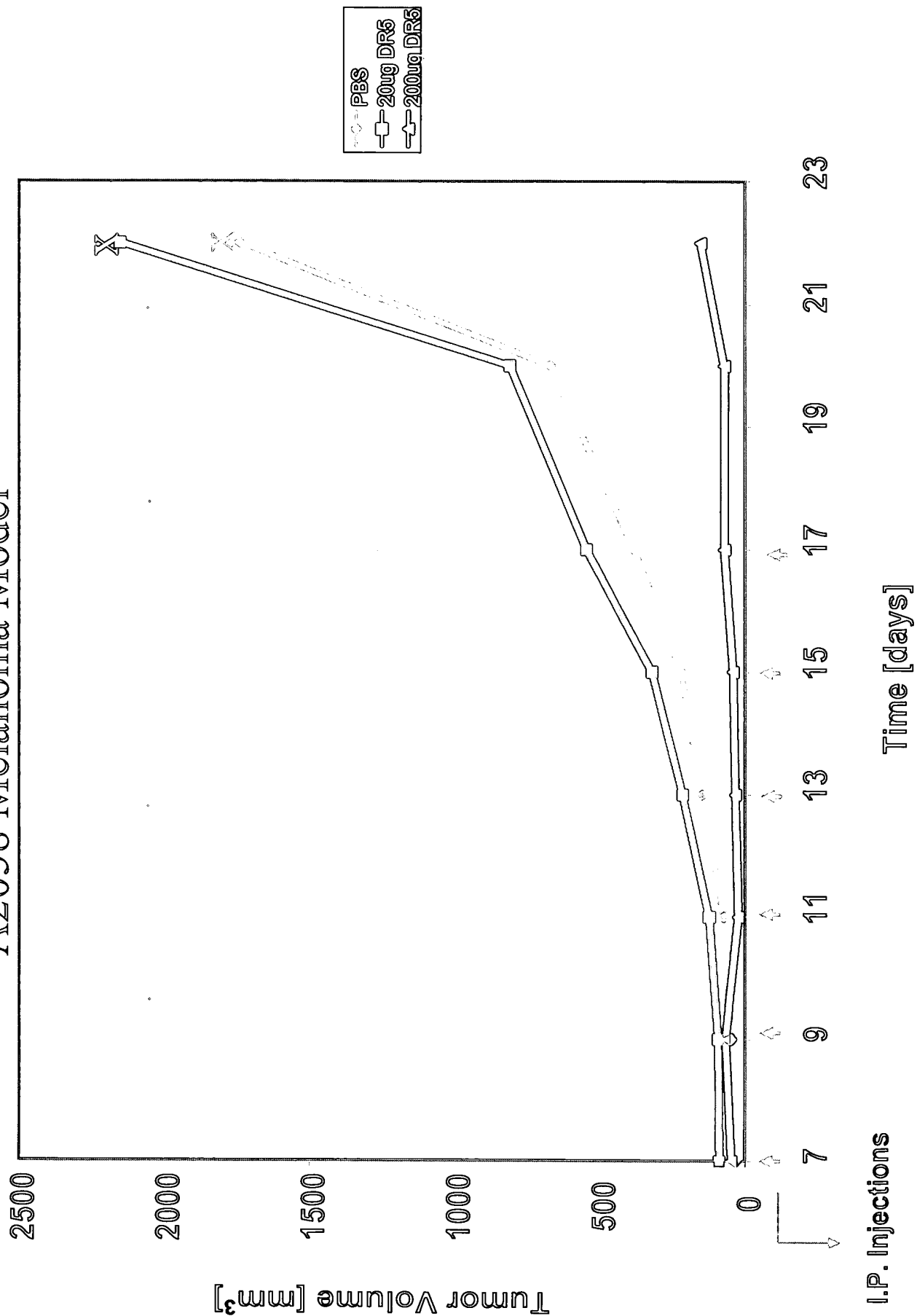
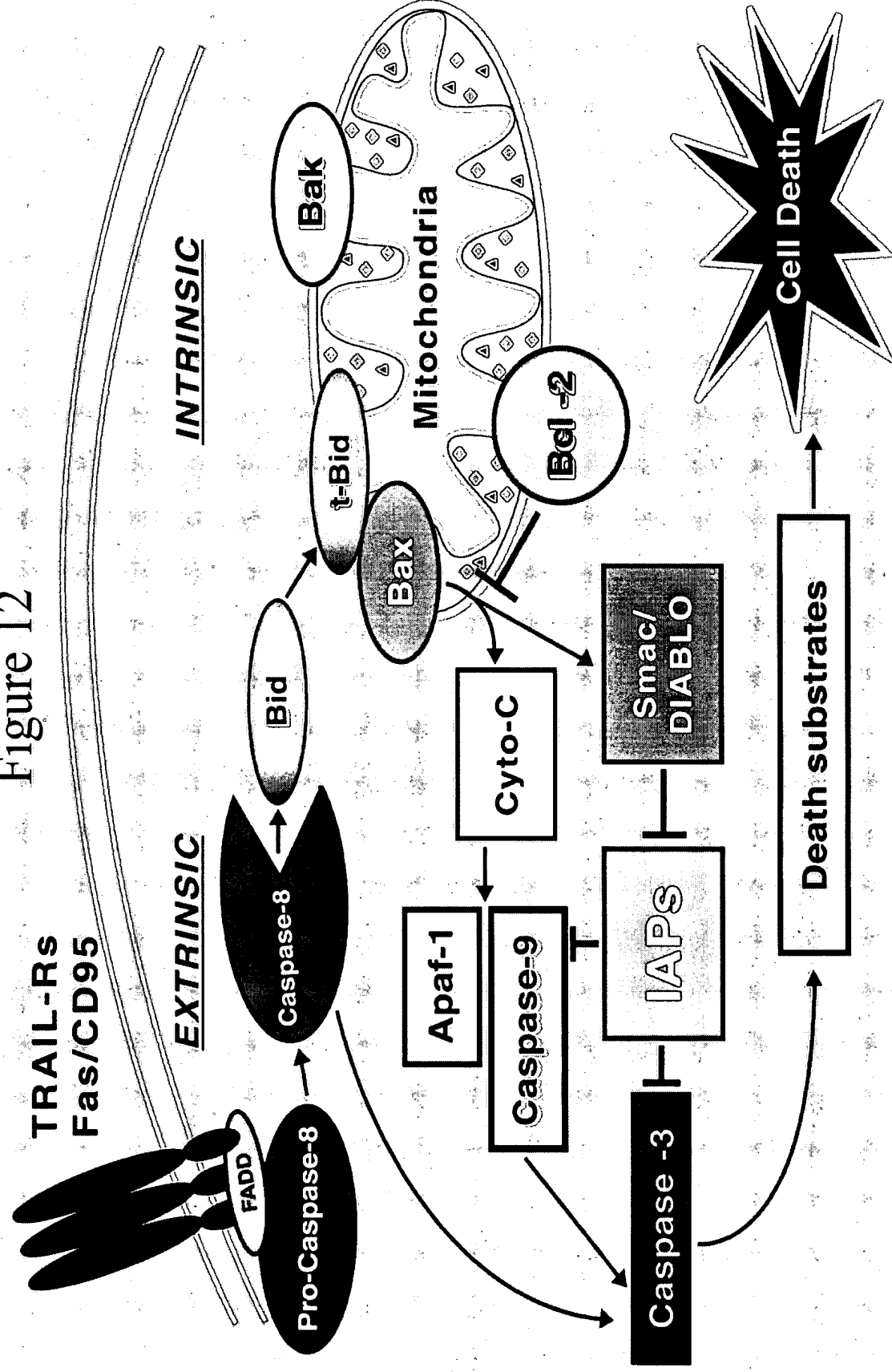


Figure 11  
Tumoricidal Activity of DR5 Monoclonal Antibody In Vivo  
A2058 Melanoma Model



# Pathways For Caspase Activation and Apoptosis

Figure 12



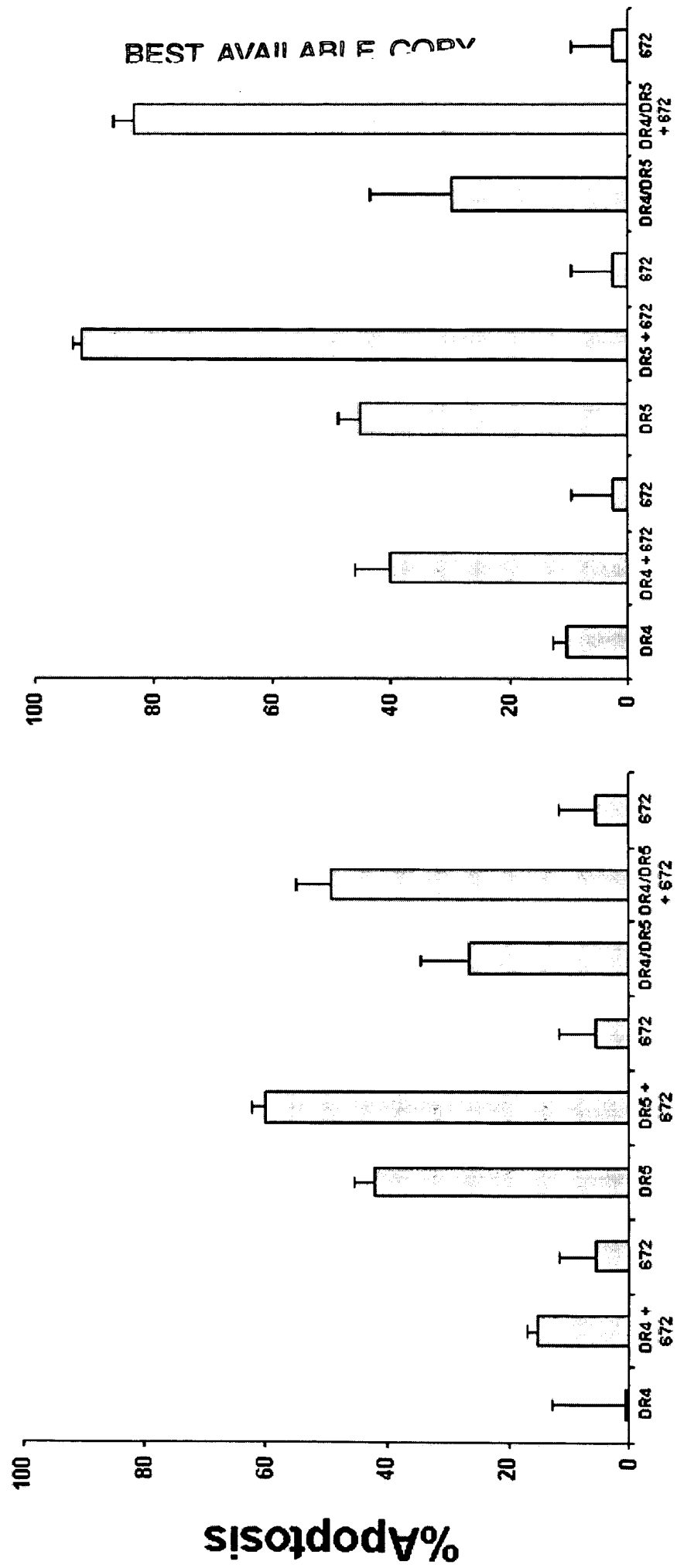
Predominant Anti-Apoptotic Features of Tumor Cells:

- Over-expression of Bcl 2
- Increased Levels of IAP's
- Mutations in Bax

# Anti-DR4 or DR5-induced apoptosis in A2058 cells in the absence or presence of 0.5 $\mu$ M LBP 672

Figure 13

24hr 48hr



# Figure 14

## Effect of LB 672 On Normal And Tumor Cells

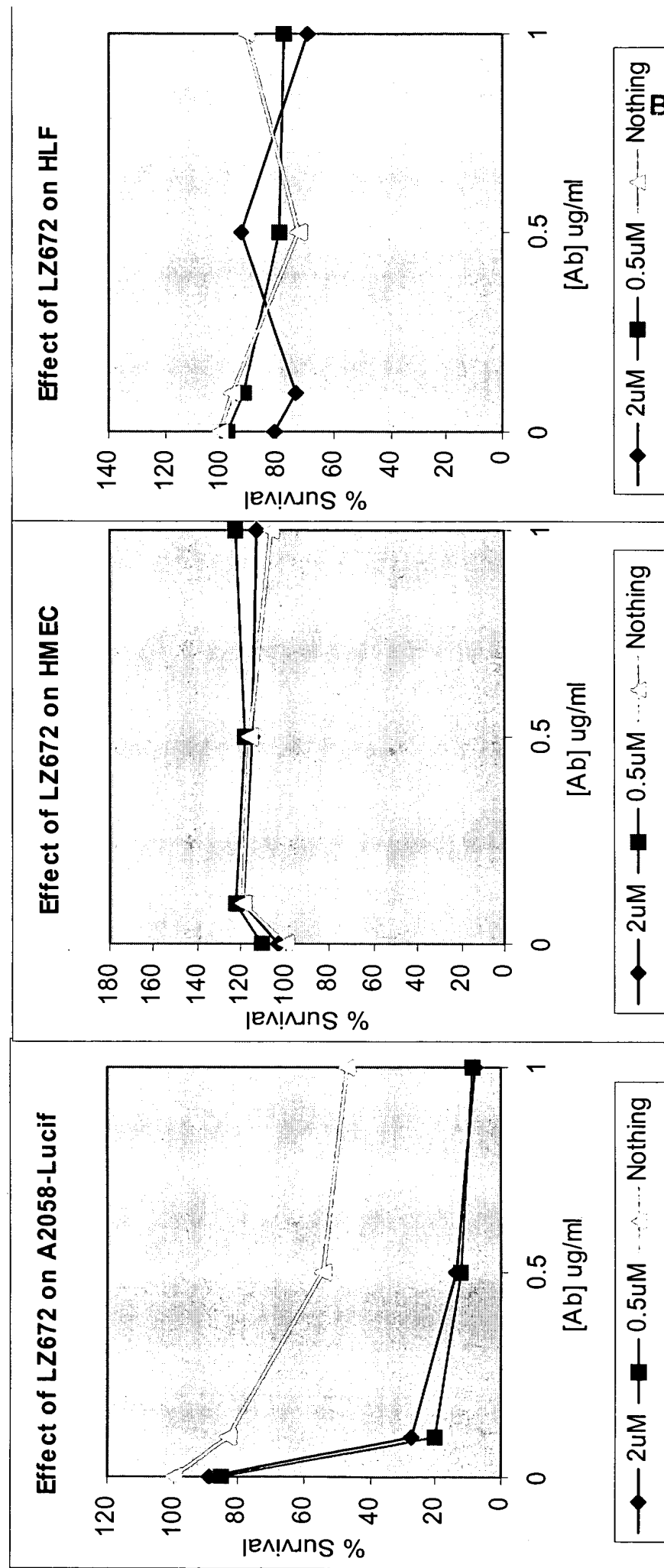
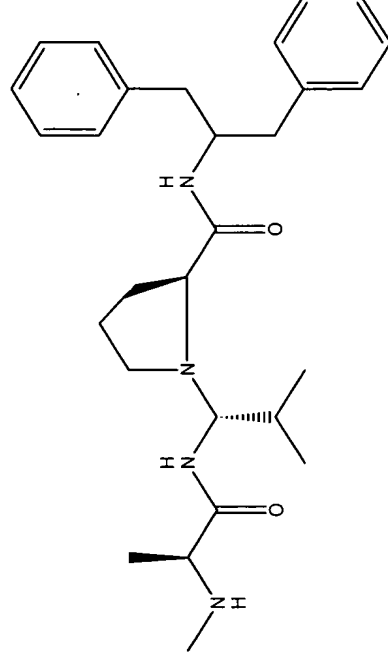
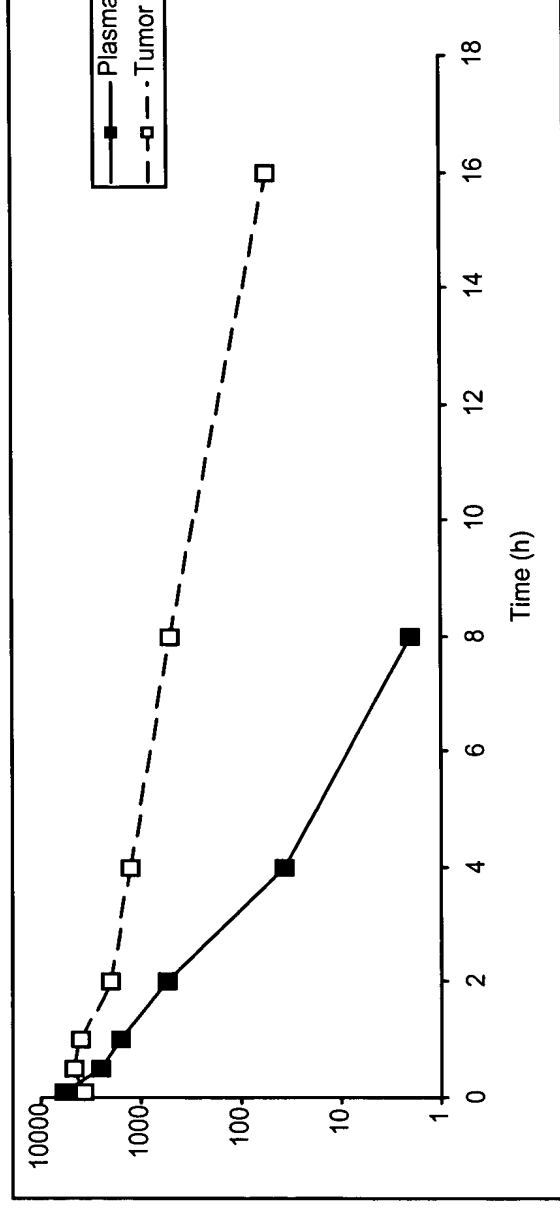


Figure 15

# PK and PD Study of Smac Mimetic LBP672 In Nude Mice Bearing HCT116 Tumors



NVP-LBP672

M.W. = 492.67 as a free base

## PK SUMMARY

Model: Athymic nude mice bearing subcutaneous HCT116 tumors

Dose: single 25 mg/kg i.v. trifluoroacetate salt (20.3 mg/kg free base) in D5W.

LBP672 rapidly absorbed by tumor Tmax @ 0.5 h. Mean tumor Cmax @ 4620ng/g (9.38 uM).

At 16 h. post dose LBP672 mean tumor [co] @ 55.1 ng/g (110 nM).

Figure 16

# NFkB Activation By The Proteasome

*Viruses, growth factors, radiation or chemotherapeutic drugs activate pathways that lead to the degradation of IκB by the proteasome. NFκB activates transcription of genes that protect the cell from apoptosis*

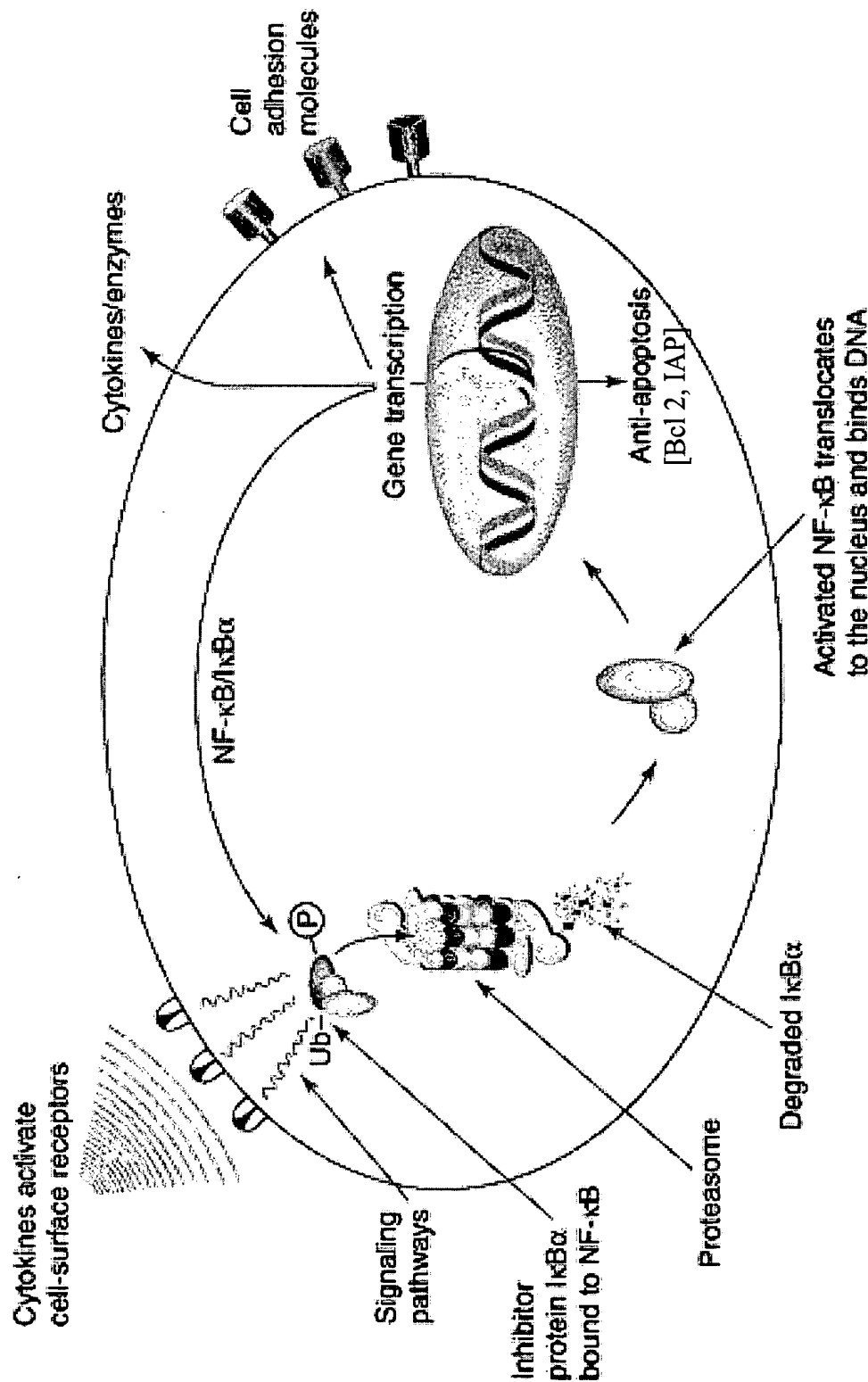




Figure 17

# Proteasome Inhibitor MG132 Enhances DR5 Antibody Induced Apoptosis of SW 480 Human Colon Carcinoma Cells

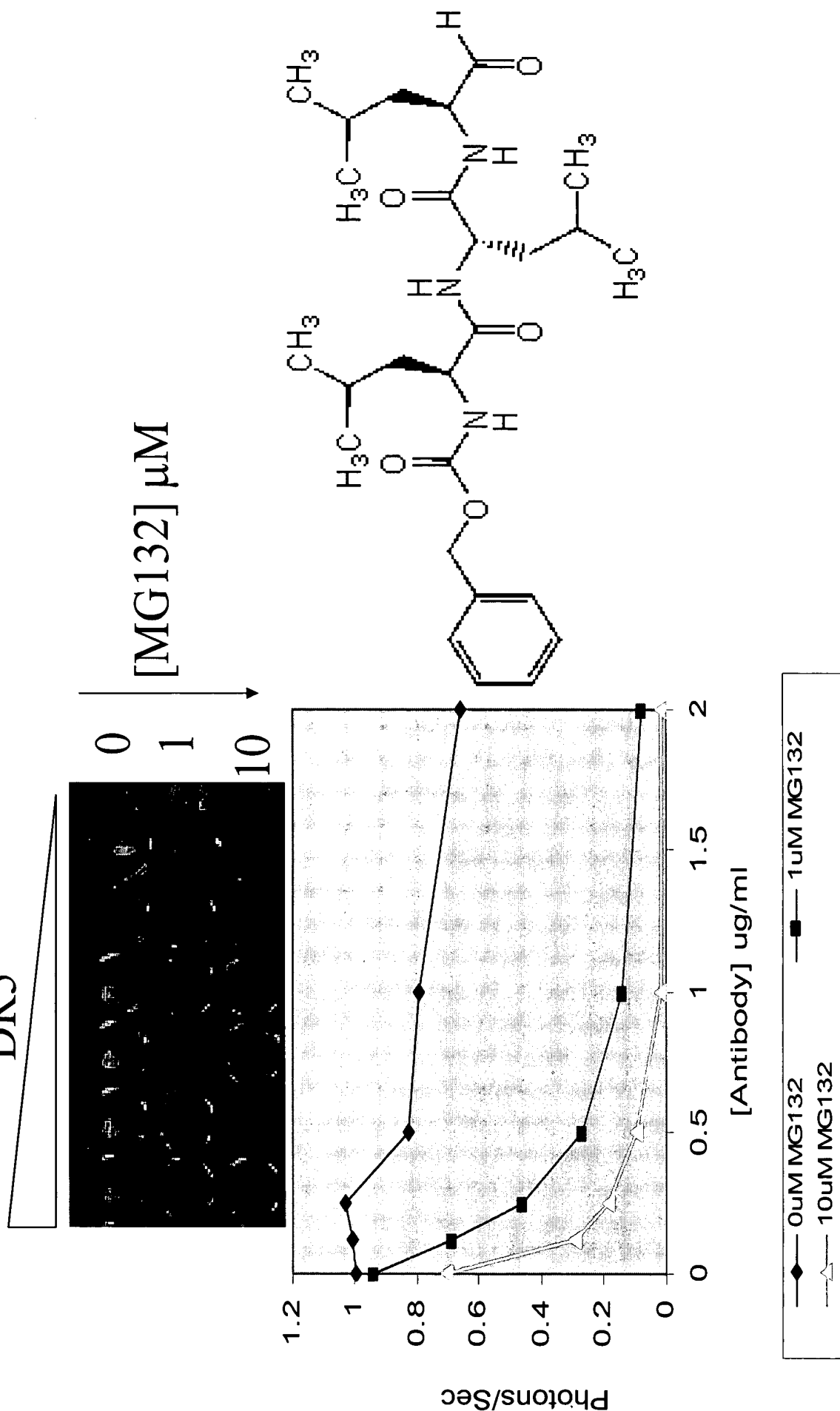


Figure 18

# 20S Proteasome Inhibitors – [Chymotryptic Activity]

Compound	Description	Selectivity	IC50	MTD	Sol.
NVP-AFB340-NX	Boronate	Good	<1nM	3mg/kg-1x	Good
NVP-AFD314-NX	Boronate	Good	<1nM	3mg/kg-1x	Good
NVP-AEV273-NX	Beta-Lactame	Excellent	3nM	20 mg/kg-2x	Poor

## NVP-AFB340-NX

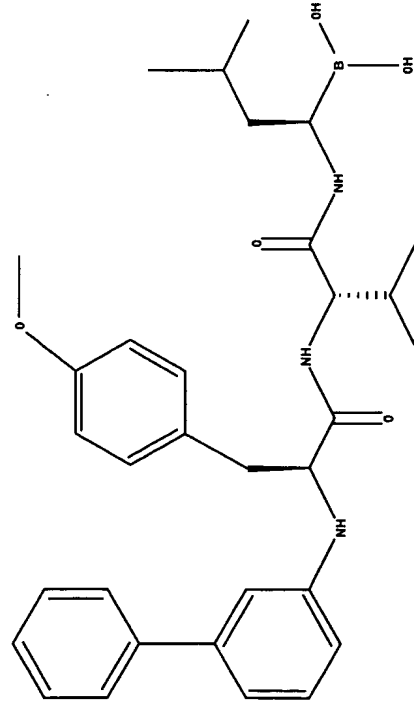


Figure 19

Effect of Proteasome Inhibitors On A2058 -Luc

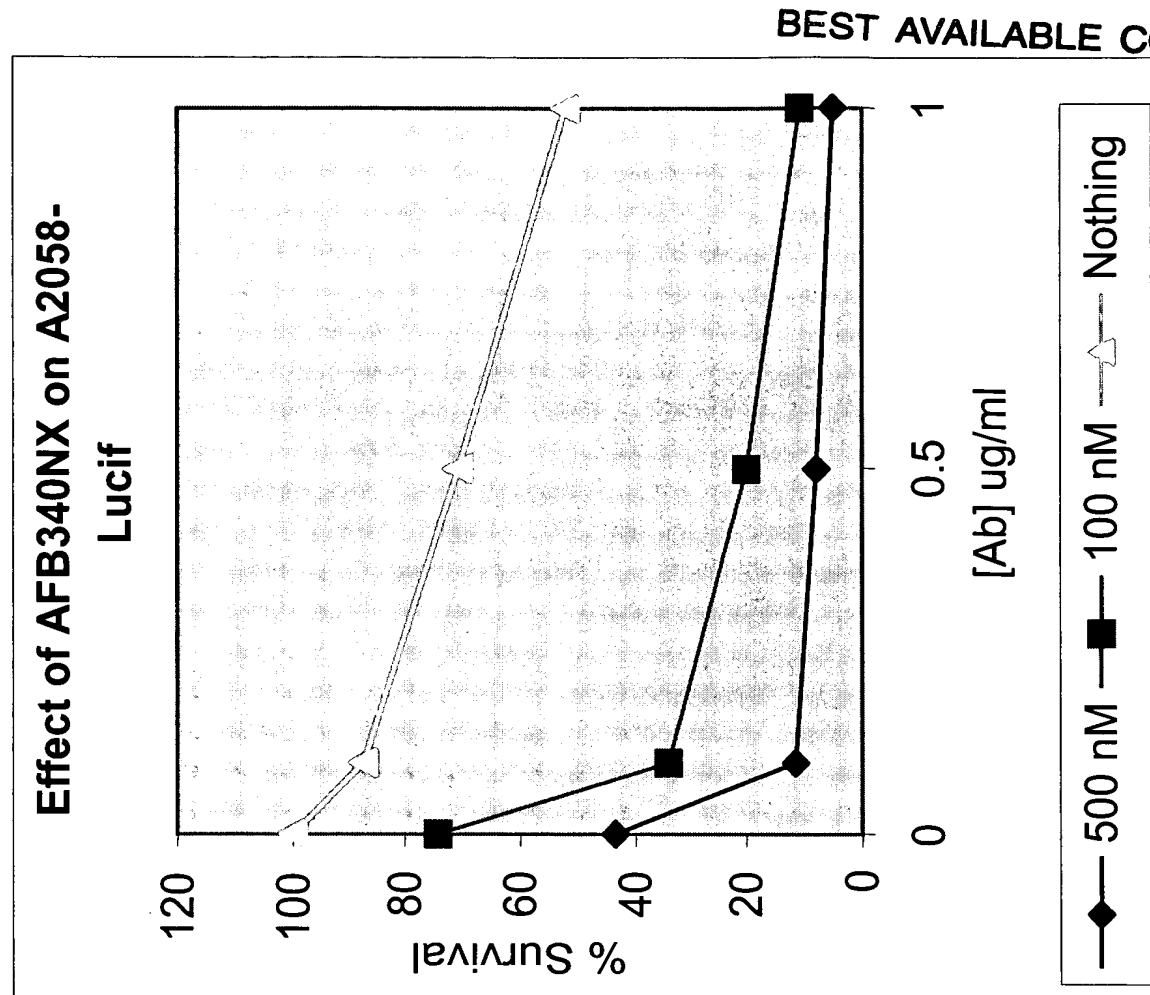
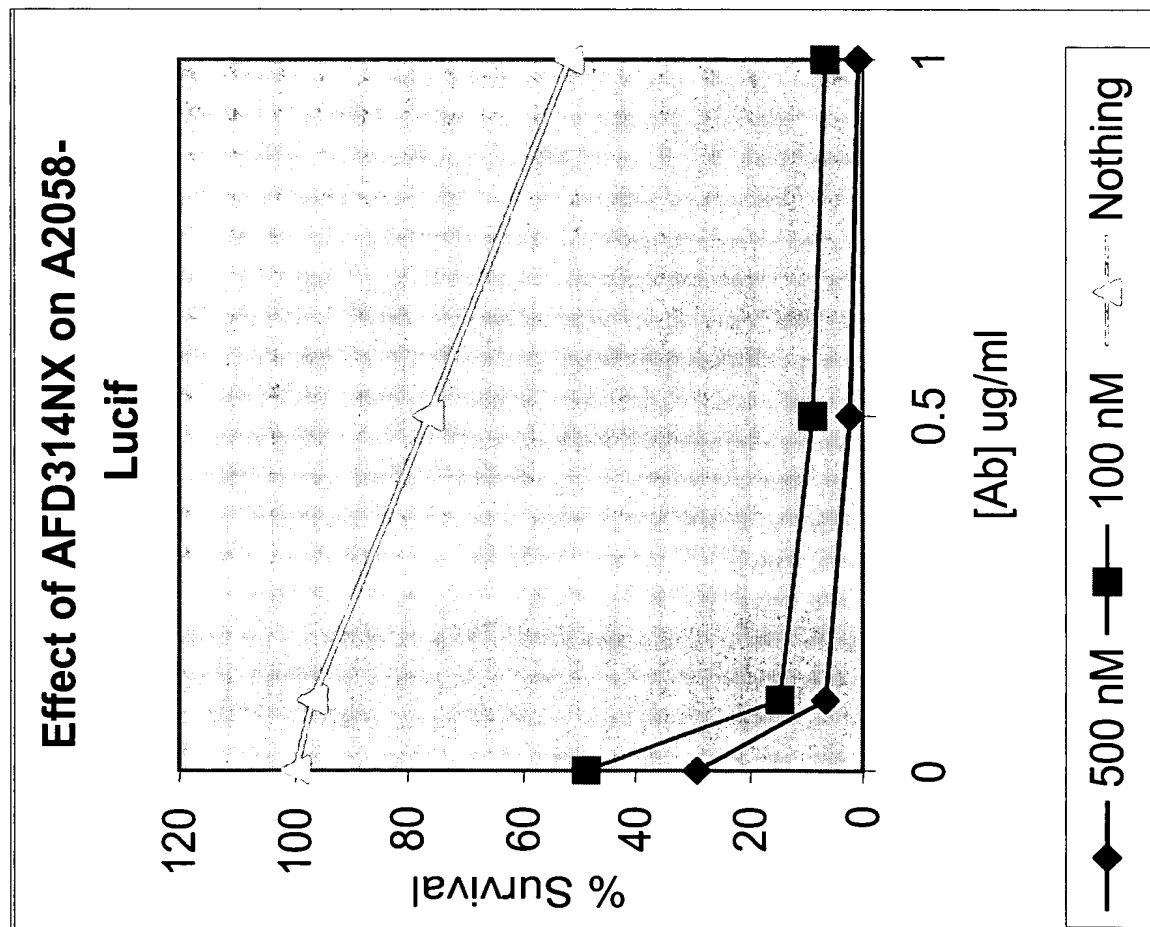


Figure 20

# Effect of Proteasome Inhibitors On The Hepatocarcinoma

## Cell Line HUH7-Luc

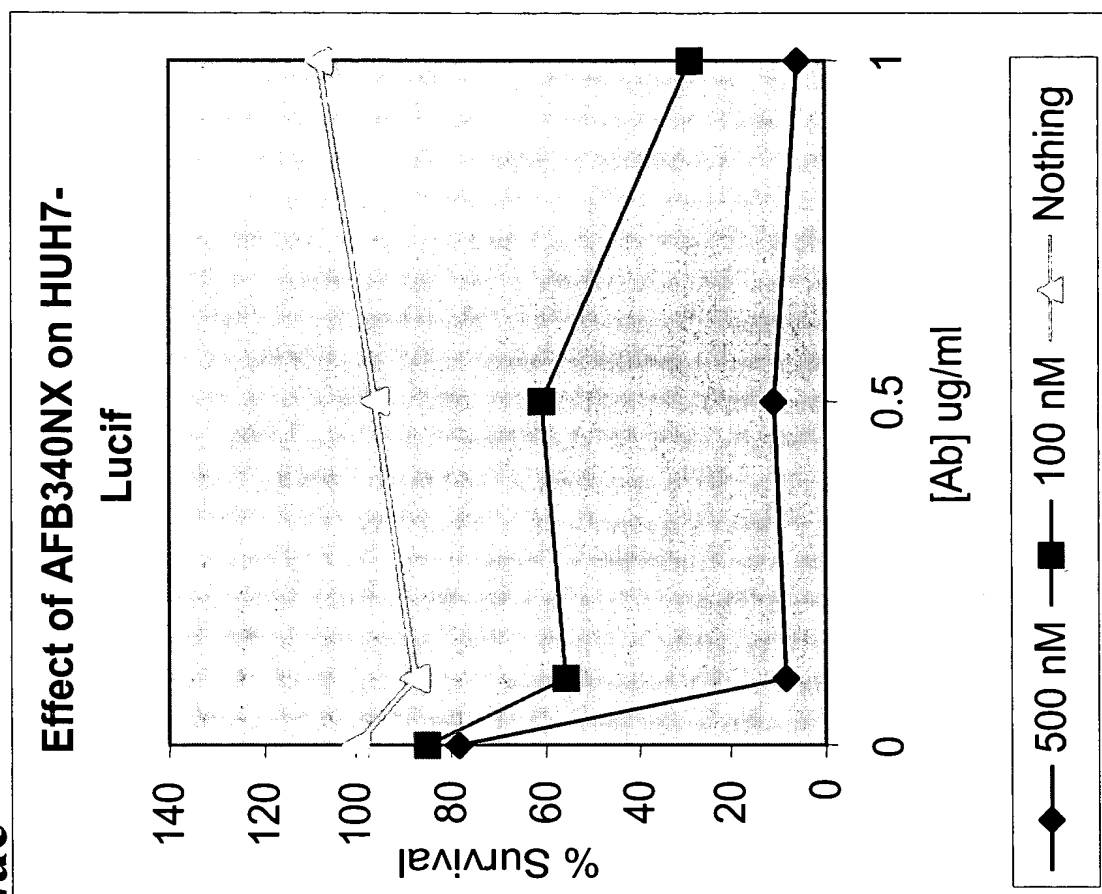
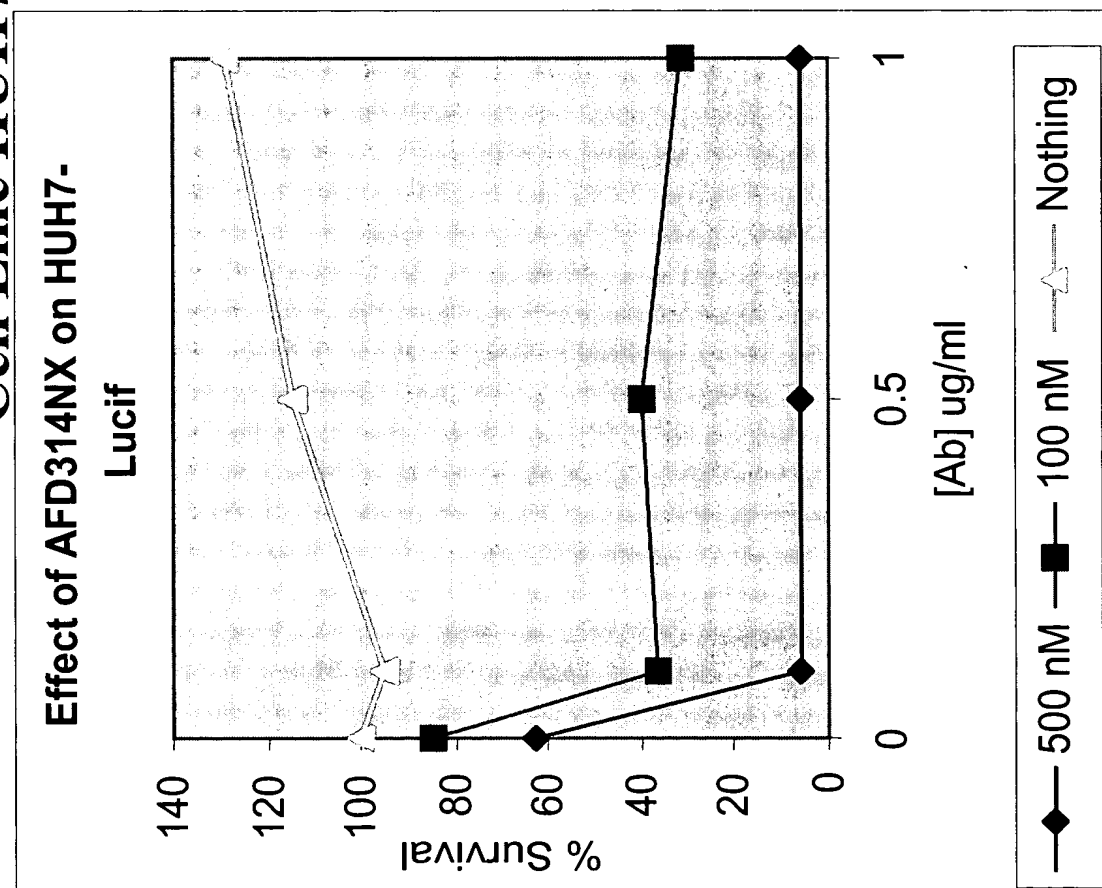


Figure 21

**Effect of Proteasome Inhibitors On Normal Human Mammary Epithelial Cells (HMEC)**

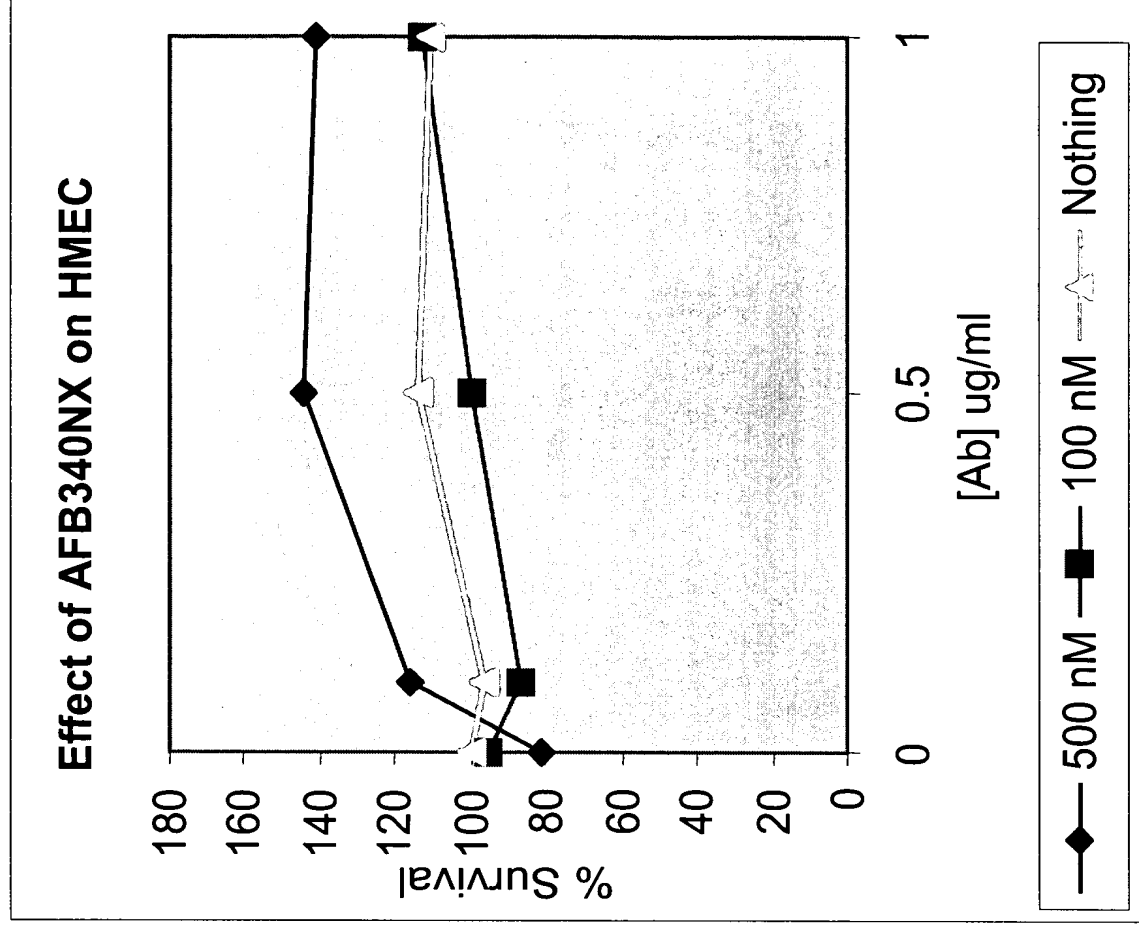
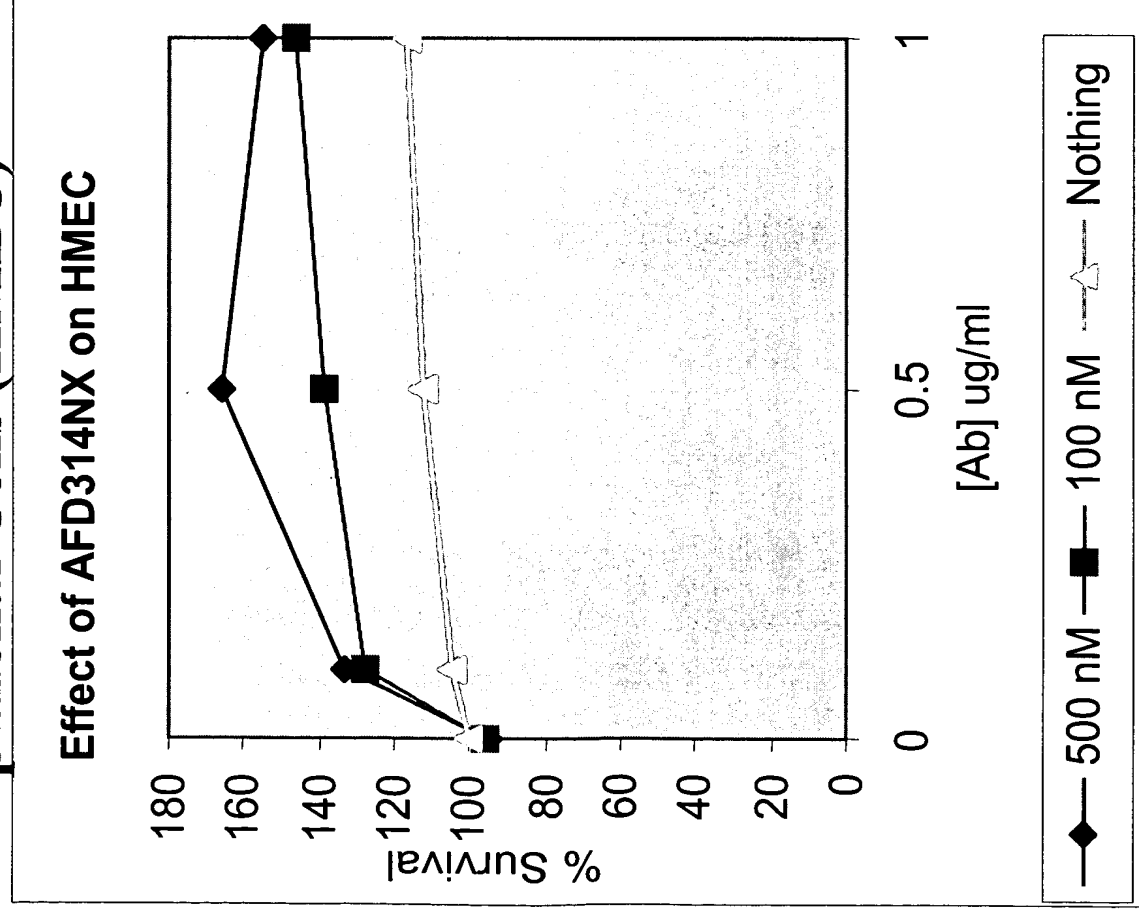
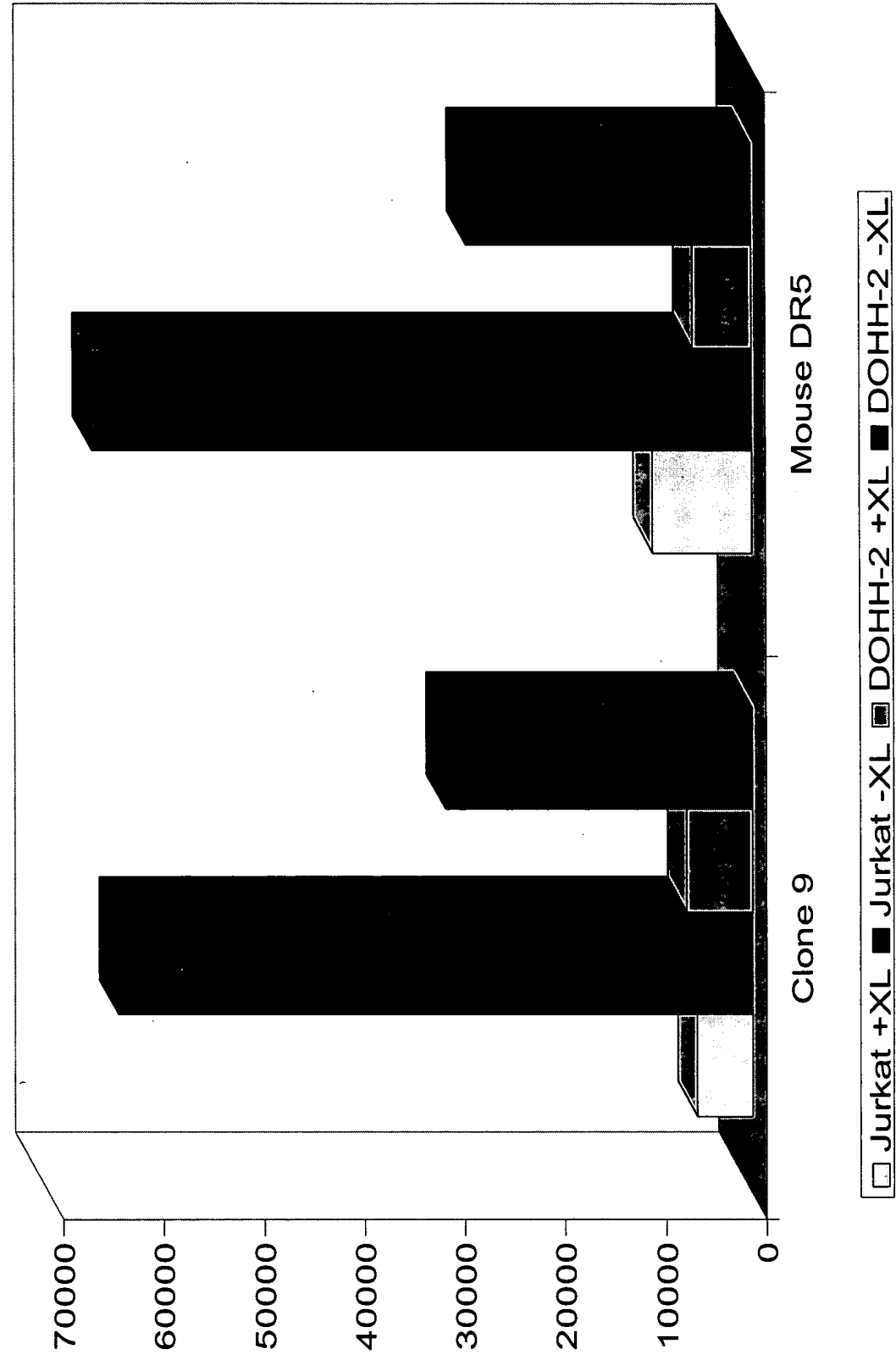


Figure 22

Functional Activity



## Figure 23

### Anti-DR5 DNA Sequence

#### Light Chain Variable Region

GACATTGCGATGACCCAGTCTCACAAAGTTTCATGTCCACATTAGTGGGAGACAGGGTCA  
GCATCACCTGCAAGGCCAGTCAGGATGTGAATACTGCTATAGCCTGGTATCAACAATAA  
ACCAGGGCAATCTCCTAAACTACTGATTTACTGGGCATCCACCCGGCACACTGGAGTC  
CCTGATCGCTTCACAGGCAGTGGATCTGGGACAGATTATACTCTCACCATCAGCAGTA  
TGGAGGCTGAAGATGCTGCCACTTATTACTGCCAGCAGTGAGTAGTAACCCGCTCAC  
GTTCCGGTGTGGACCAAGCTGGAGCTGAACGGGCTGATGCTGCACCAACTGTATCC  
ATCTTCCCACC

#### Heavy Chain Variable Region

CAGGCAAAGGTCCAGCTGCAGCAGTCTGGAGCTGAGCTGGTGAAACCCGGGCATCA  
GTGAAGCTGTCCTGCAAGGCTTCTGGCTACACCTTCACTGACTATACTATACTGGGT  
AAAGCAGAGGTCTGGACAGGGTCTTGAGTGGAATTGGGTGTTTACCCTGGAGGTGGT  
TATATAAAATACAAATGAGAAATTCAAGGACAGGGCCACATTGACTGCGGACAAATCCT  
CCAACACAGTCTATATGGAGCTTAGTCGATTGACATCTGAAGGCTCTGCGGTCTATTTC  
TGTGCAAGACACGAAGAGGGCATCTATTTTGACTACTGGGGCCAAGGCACCACTCTCA  
CAGTCTCCTCA

Figure 24

## DR5 V<sub>H</sub> Sequence – Heavy Chain Subgroup 2B

### *FR1*

Lys Val Gln Leu Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala Ser Val

### *CDR1*

Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr Thr Ile His Trp Val

### *FR2*

Lys Gln Arg Ser Gly Gln Gly Leu Glu Trp Ile Gly Trp Phe Tyr Pro Gly Gly

### *CDR2*

Gly Tyr Ile Lys Tyr Asn Glu Lys Phe Lys Asp Arg Ala Thr Leu Thr Ala Asp

### *FR3*

Lys Ser Ser Asn Thr Val Tyr Met Glu Leu Ser Arg Leu Thr Ser Glu Gly Ser

### *CDR3*

Ala Val Tyr Phe Cys Ala Arg His Glu Glu Gly Ile Tyr Phe Asp Tyr Trp Gly

### *FR4*

Gln Gly Thr Thr Leu Thr Val Ser Ser



Figure 25

## DR5 V<sub>L</sub> Sequence– Kappa Light Chain Subgroup 5

### *FR1*

Asp Ile Ala Met Thr Gln Ser His Lys Phe Met Ser Thr Leu Val Gly Asp

### *CDR1*

Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp Val Asn Thr Ala Ile Ala

### *FR2*

Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Ile Tyr Trp Ala

### *CDR2*

### *FR3*

Ser Thr Arg His Thr Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly

Thr Asp Tyr Thr Leu Thr Ile Ser Ser Met Glu Ala Glu Asp Ala Ala Thr

### *CDR3*

### *FR4*

Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Leu Thr Phe Gly Ala Gly Thr

Lys Leu Glu Leu Lys Arg Ala

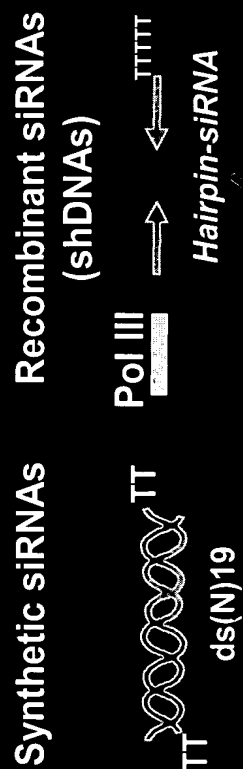
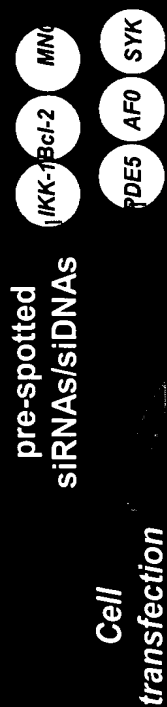


Figure 26



Assays:

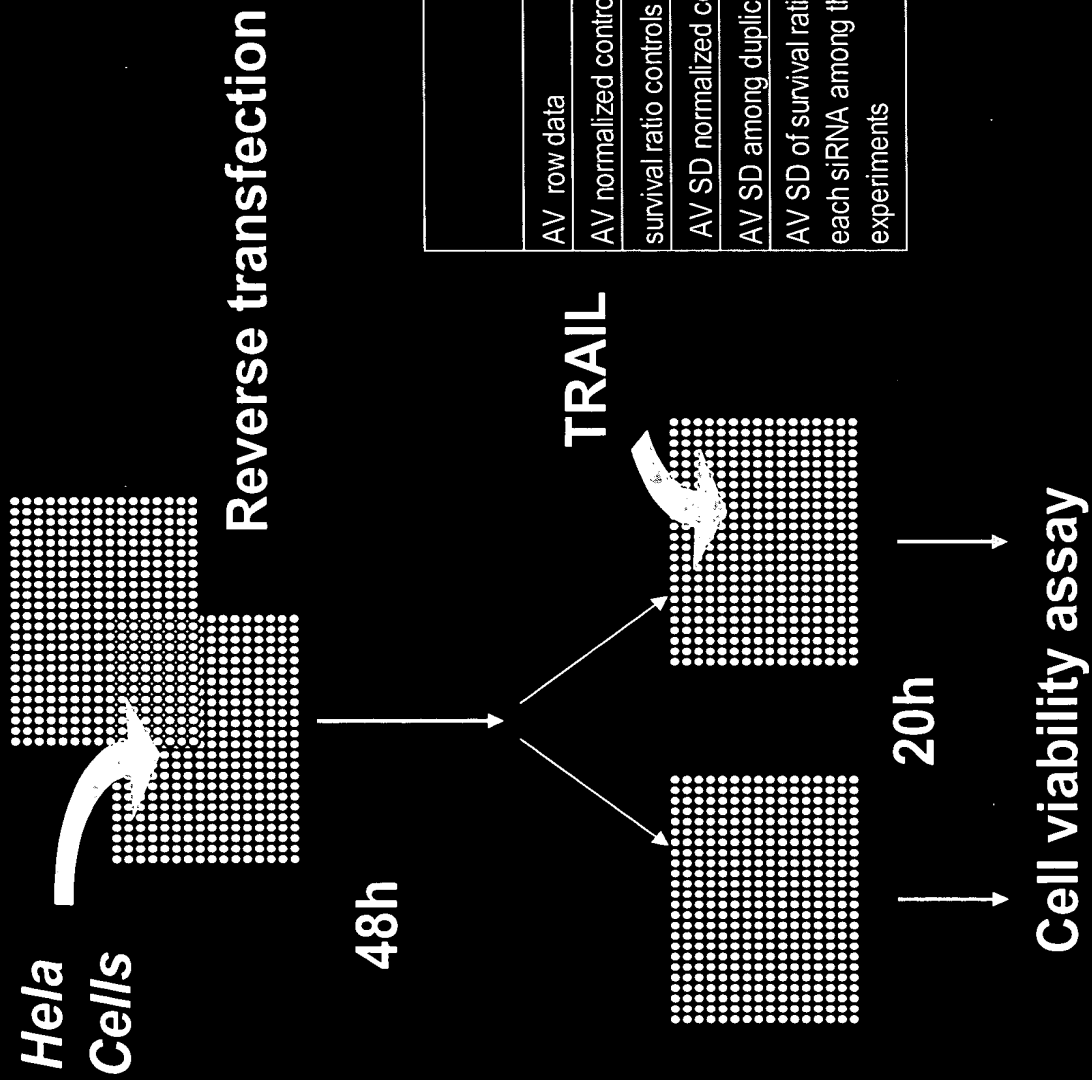
Viability, cell survival

Reporter assays

Image based



Figure 27  
TRAIL induced apoptosis in Hela cells



	screen 1		screen2	
	(-TRAIL)	(+TRAIL)	(-TRAIL)	(+TRAIL)
AV row data	29221.7	9368.75	59419.2	26782.7
AV normalized controls	100	32	100	45
survival ratio controls	0.32		0.45	
AV SD normalized controls	7.06	16.12	6.18	10.76
AV SD among duplicates	5.75	8.02	4.11	6.71
AV SD of survival ratio for each siRNA among the 2 experiments	12.87			

Screen

1 2

TRAIL enhancers

siRNA

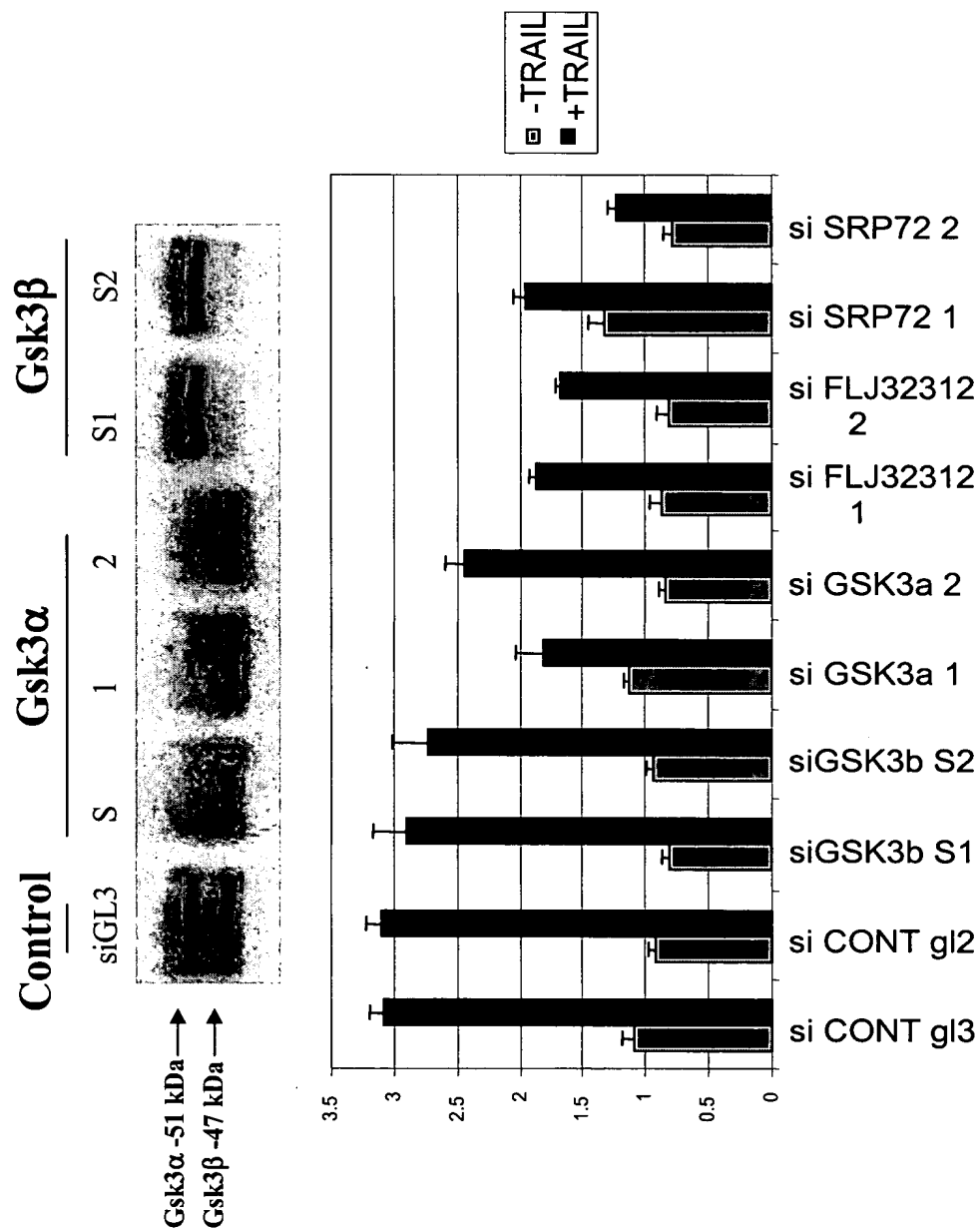
TRAIL inhibitors

Log(ratio)

Figure 28

ratio	P value	annotation
8.09	0.000	H.s. plexin B1 (PLXNB1), mRNA <sup>""</sup>
9.24	0.000	H.s. SET domain-containing protein 7 (SET7), mRNA
9.89	0.001	H.s. mitogen-activated protein kinase kinase kinase 5 (MAP3K5), mRNA <sup>""</sup>
10.22	0.001	H.s. STE20-like kinase (JIK), mRNA
10.46	0.001	H.s. putative endoplasmic reticulum multispan transmembrane protein (RFT1), mRNA <sup>""</sup>
10.47	0.001	H.s. MAP kinase-interacting serine/threonine kinase 1 (MKNK1), mRNA <sup>""</sup>
10.70	0.001	H.s. mitogen-activated protein kinase-activated protein kinase 2 (MAPKAPK2), trans
11.29	0.002	Homo sapiens phosphatidylinositol-4-phosphate 5-kinase, type I, gamma (PIP5K1C
11.88	0.004	MAP2k5
12.31	0.005	Q62862 SER/THR FAMILY OF PROTEIN KINASES-RELATED
12.51	0.006	H.s. cyclin-dependent kinase 6 (CDK6), mRNA <sup>""</sup>
12.73	0.008	H.s. muscle, skeletal, receptor tyrosine kinase (MUSK), mRNA <sup>""</sup>
12.76	0.007	H.s. activin A receptor type II-like 1 (ACVRL1), mRNA <sup>""</sup>
12.92	0.008	H.s. Gardner-Rasheed feline sarcoma viral (v-gr) oncogene homolog (FGR), mRNA <sup>""</sup>
13.16	0.011	H.s. ribosomal protein S6 kinase, 90kD, polypeptide 5 (RPS6KA5), mRNA <sup>""</sup>
13.26	0.007	H.s. hypothetical protein FLJ21802 (FLJ21802), mRNA (mina53 related)
13.95	0.018	H.s. mitogen-activated protein kinase 7 (MAPK7), mRNA
74.00	0.008	H.s. glycogen synthase kinase 3 alpha (GSK3A), mRNA <sup>""</sup>
75.07	0.010	hypothetical protein FLJ32312 (FLJ32312),
77.75	0.007	similar to Pyruvate kinase, M2 isozyme (LOC148283),
78.62	0.004	H.s. hypothetical protein FLJ11712 (FLJ11712), mRNA
80.10	0.004	H.s. B lymphoid tyrosine kinase (BLK), mRNA <sup>""</sup>
84.64	0.003	P53
94.56	0.001	Hs. signal recognition particle 72kD (SRP72), mRNA

Figure 29



# Figure 30

Global analysis

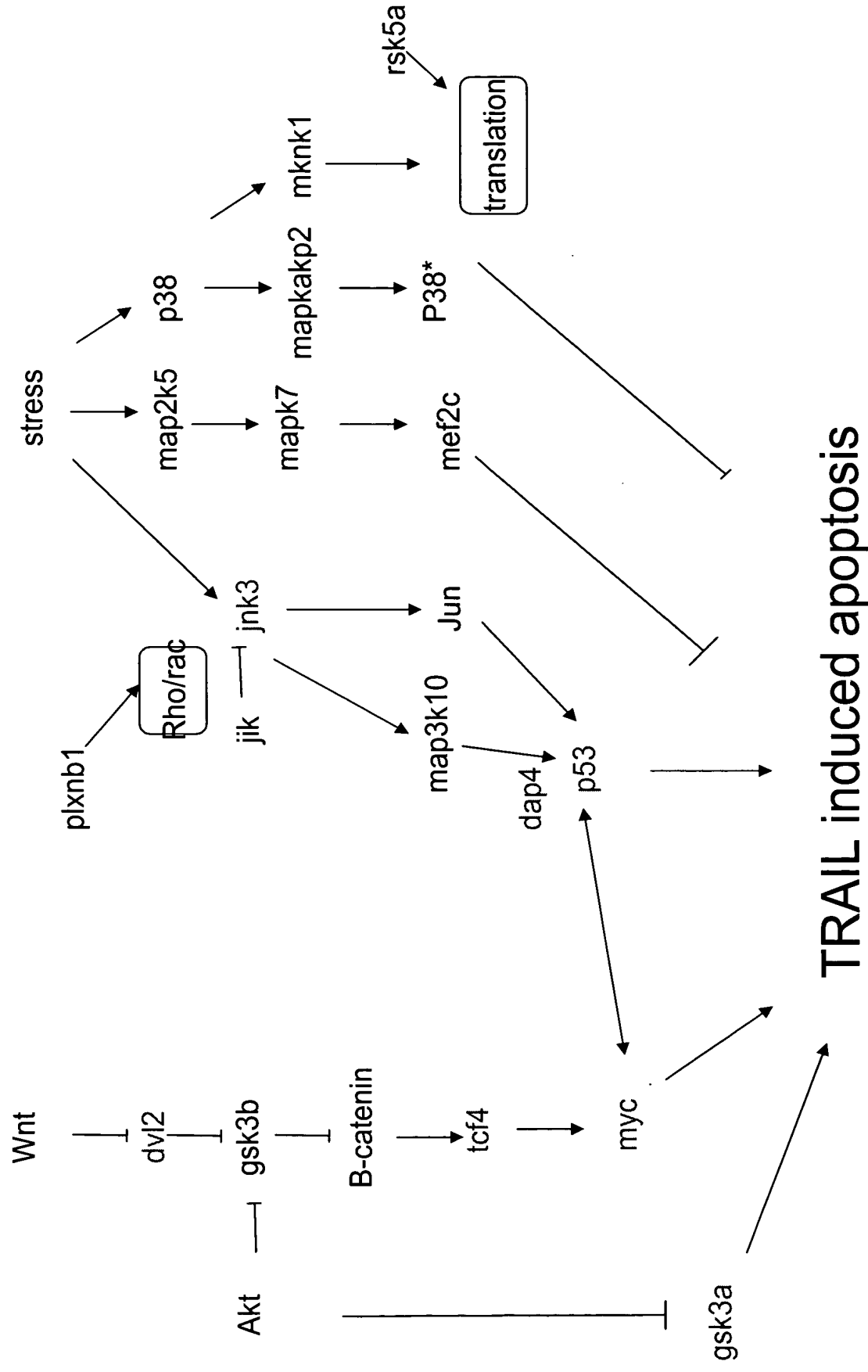
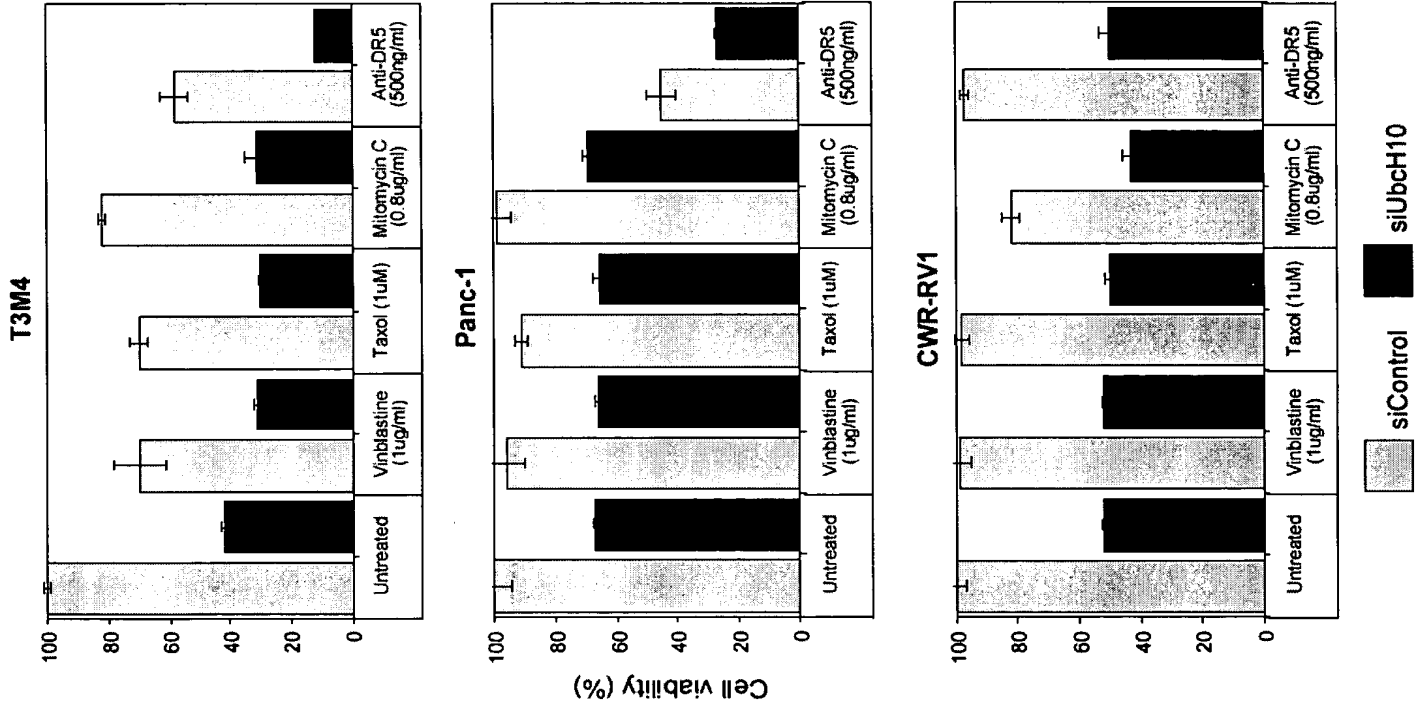


Figure 31



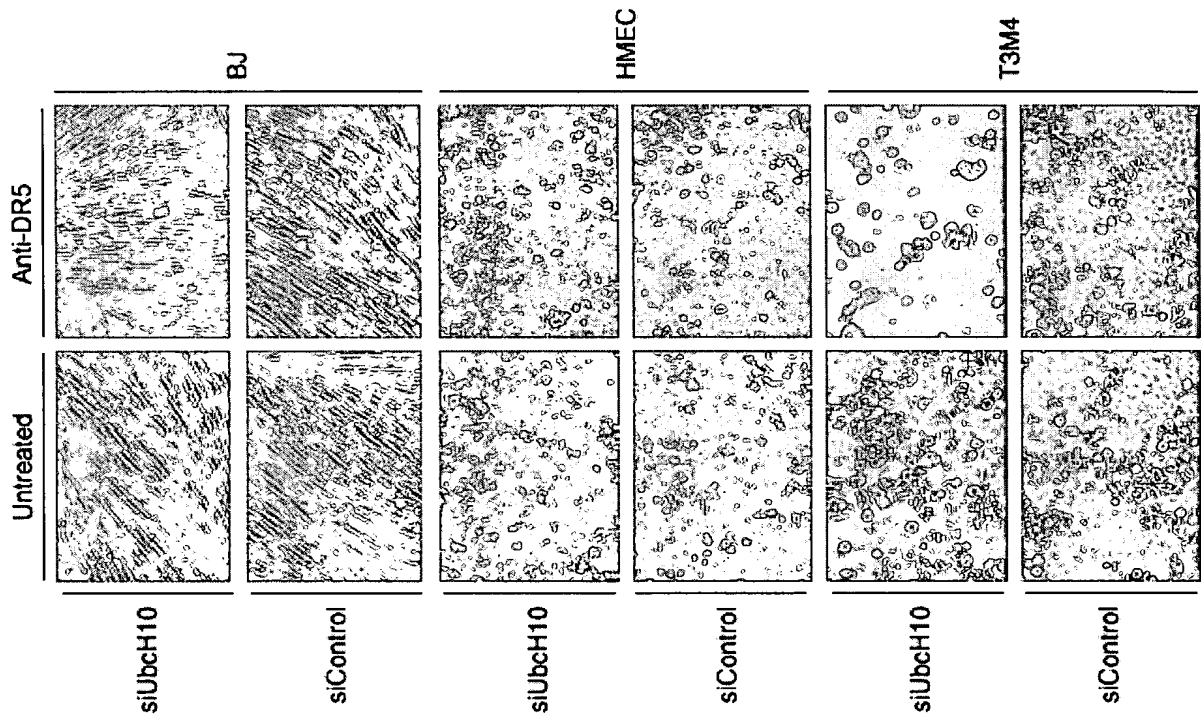


Figure 32



# Sensitization of HcT116-Bax<sup>-/-</sup> to TRAIL by inhibition of the proteasome

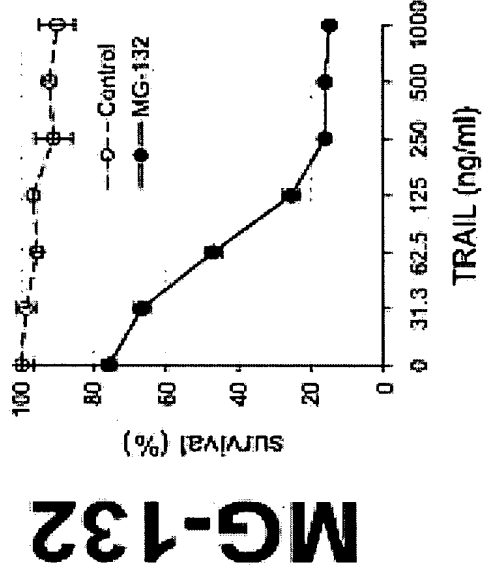
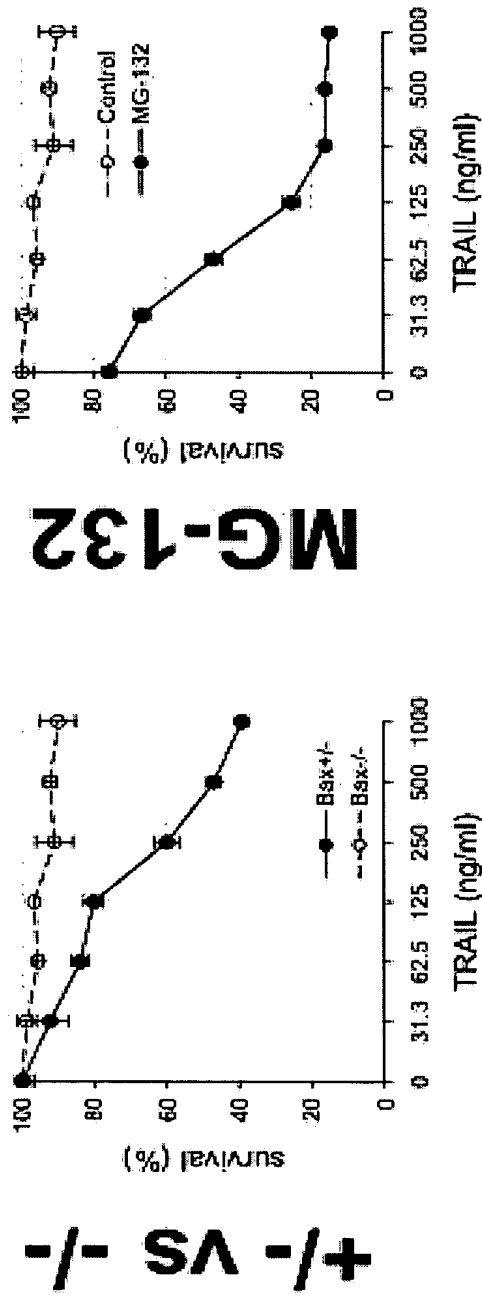
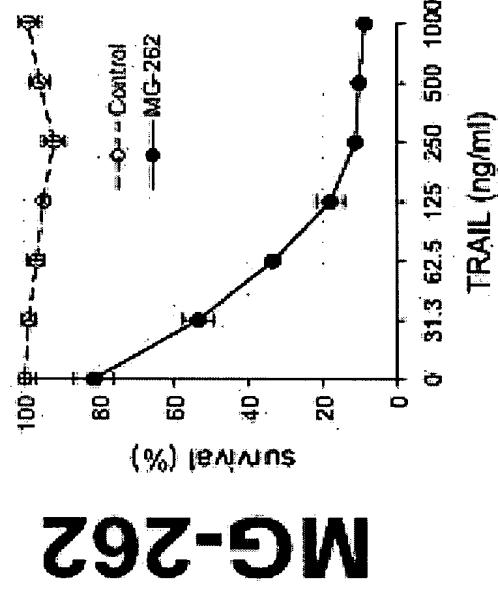
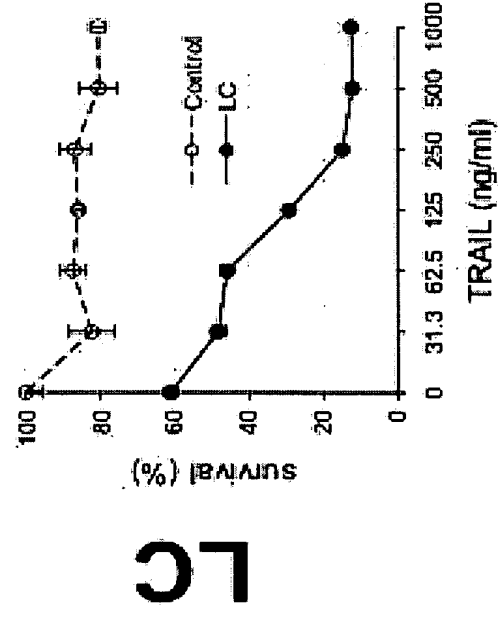
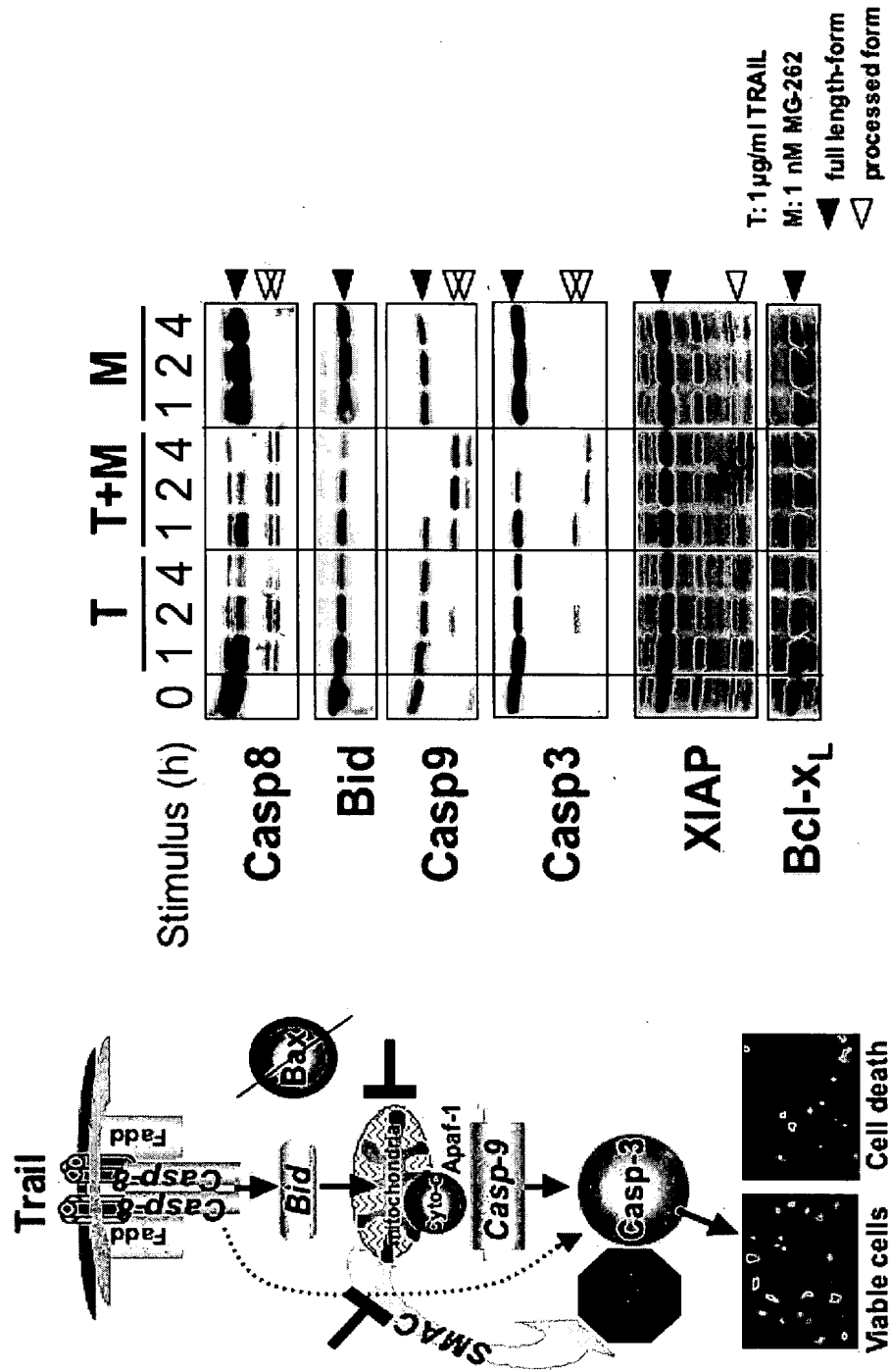


Figure 33



Measurement after 24 h, 5  $\mu$ M LC, 1  $\mu$ M MG-132, 1 nM MG-262

**Figure 34**  
**MG-262 restores the mitochondrial**  
**apoptosis pathway**



## Figure 35

### Sequence of DR5'A' heavy chain variable region

AAGTCCAGCTGCAGCAGTCTGGAGCTGAGCTGGTGAACCCGGGCATCAGTGAA  
GCTGTCCTGCAAGGCTTCTGGCTACACCTTCACTGACTATACTATACACTGGGTAAA  
GCAGAGGTCTGGACAGGGTCTTGAGTGGATTGGGTGTTTACCCTGGAGGTGGTTA  
TATAAAATACAAATGAGAAATTCAAGGACAGGGCCACATTGACTGCGGACAAATCCTC  
CAACACAGTCTATATGGAGCTTAGTCGATTGACATCTGAAGACTCTGCGGTCTATTTC  
TGTGCAAGACACGAAGAGGGCATCTATTTTGACTACTGGGGCCAAGGCACCACTCTC  
ACAGTCTCCTCA

### Amino acid sequence of VH

KVQLQQSGAELVKPGASVKLSCKASGYTFTDYTIHWVKQRSQGLEWIGWIFYPGGGYIK  
YNEKFKDRATLTADKSSNTVYMELSRLTSEDSAVYFCARHEEGIFYDWGQGTTLTVSS

### DNA Sequence of DR5'A' light chain variable region

GACATTGTGATGACCCAGTCTCACAAGTTCATGTCCACATCAGTGGGAGACAGGGTCA  
GCATCACCTGCAAGGCCAGTCAGGATGTGAATACTGCTATAGCCTGGTATCAACAAA  
ACCAGGGCAATCTCCTAACTACTGATTACTGGGCATCCACCCGCACACTGGAGTC  
CCTGATCGCTTCACAGGCAGTGGATCTGGGACAGATTATACTCTACCATCAGCAGTGT  
GCAGGCTGAAGACCTGGCACTTTATTACTGTCAGCAACATTATACCACTCCATTACAGT  
TCGGCTCGGGGACAAAGTTG

### Amino acid sequence of VL

DIVMTQSHKFMSTSVGDRVSITCKASQDVNTAIAWYQQKPGQSPKLLIYWASTRHTGVPDR  
FTGSGSGTDYTLTISSVQAEDLALYCCQQHYTTPFTFGSGTKL